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FORM PTO-1390
(REV 10-95)

U.S. DEPARTMENT OF COMMERCE AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER
CFV-005.01

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
09/171553

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB97/01087	18 April 1997 (18.04.97)	19 April 1996 (19.04.96)

TITLE OF INVENTION:
PORCINE RETROVIRUS

APPLICANT(S) FOR DO/EO/US
Todd CAMPUS and Douglas HOUSE

10/19/98

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. (X) This is the FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ( ) This a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. (X) This express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. (X) A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. (X) A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ( ) is transmitted herewith (required only if not transmitted by the International Bureau).
   b. (X) has been transmitted by the International Bureau.
   c. ( ) is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ( ) A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ( ) Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ( ) are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ( ) has been transmitted by the International Bureau.
   c. ( ) have not been made; however, the time limit for making such amendments has NOT expired.
   d. ( ) have not been made and will not be made.
8. ( ) A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ( ) An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ( ) A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ( ) An information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ( ) An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. (X) A FIRST preliminary amendment.
    ( ) A SECOND or SUBSEQUENT preliminary amendment.
14. ( ) A substitute specification.
15. ( ) A change of power of attorney and/or address letter.
16. (X) Other items of information.
    (X) International Preliminary Examination Report (with translation)
    (X) PCT Request Form.

Certificate of Express Mail

I hereby certify that the foregoing documents are being deposited with the United States Postal Service as Express Mail, postage prepaid, "Post Office to Addressee", in an envelope addressed to the Assistant Commissioner for Patents, Box PCT, Attn: DO/EO/US, Washington, D.C. 20231 on the date indicated below.

Roberta L. Hahn

Roberta L. Hahn
Express Mail Label: EL046501414us
Date of Deposit: October 19, 1998

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO. PCT/GB97/01087	ATTORNEY'S DOCKET NUMBER CFV-005.01

17. (X) The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

		CALCULATIONS PTO USE ONLY
Search Report has been prepared by the EPO or JPO	**$910.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482)	**$700.00**	
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))	**$770.00**	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$1040.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)	**$96.00**	

ENTER APPROPRIATE BASIC FEE AMOUNT =	$910.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ( ) 20 (x) 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$130.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	40 - 20 =	20	X $22.00	$ 440.00	
Independent claims	9 - 3 =	6	X $80.00	$ 480.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $260.00	$ 260.00	
	TOTAL OF ABOVE CALCULATIONS =			$2220.00	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				$0	
SUBTOTAL =				$2220.00	
Processing fee of **$130.00** for furnishing the English translation later than ( ) 20 ( ) 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$0	
TOTAL NATIONAL FEE =				$2220.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property. +				$0	
TOTAL FEES ENCLOSED =				$2220.00	
				Amount to be: refunded	$
				charged	$

a. (X) A check in the amount of $2220.00 to cover the above fees is enclosed.

b. ( ) Please charge my Deposit Account No **06-1448,** in the amount of **$0** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. (X) The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **06-1448, Ref. CFV-005.01.** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Patent Group
Foley, Hoag & Eliot LLP
One Post Office Square
Boston, MA 02109-2170

SIGNATURE

Chinh H. Pham

REGISTRATION NO. 39,329

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Todd CAMPUS et al.	Atty Docket:	CFV-005.01
Serial No.:	Not Yet Assigned	Art Unit:	Not Yet Assigned
Date Filed:	Herewith	Examiner:	Not Yet Assigned
Based on:	PCT/GB97/01087		
International Filing Date:	18 April 1997 (18.04.97)		
Priority Date:	19 April 1996 (19.04.96)		
Title of Invention:	*PORCINE RETROVIRUS*		

FIRST PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to consideration of the above-referenced application, amend the application as follows:

IN THE CLAIMS:

Please cancel claims 6, 7 and 25 without prejudice.

Please amend the claims as follows:

1. (Amended) An isolated polynucleotide fragment as shown in Figures 1, 2, 3 or 4, subsequence thereof, or corresponding RNA sequence thereof:

   (a) encoding at least one porcine retrovirus (PoEV) expression product;

   (b) encoding a derivative of said expression product displaying a physiological [physiologically active] and/or immunological activity [immunogenic derivative] of said expression product as described in (a); or

   (c) which is complementary to a polynucleotide sequence as defined in (a) or (b).

2. (Amended) An isolated polynucleotide fragment according to claim 1:

   (a) encoding at least one polypeptide having an amino acid sequence which is shown in Figures 3 or 4 [the polymerase (POL) polypeptide];

(b) encoding a [physiologically active and/or immunogenic] derivative of said at least one [a] polypeptide displaying a physiological and/or immunological activity substantially similar to the physiological and/or immunological activity of said expression product as described in (a); or

(c) which is complementary to a polynucleotide sequence as defined in (a) or (b).

3. (Amended) An isolated polynucleotide fragment according to claim 1 or 2:

(a) encoding the polymerase (POL) [virion core polypeptide (GAG) and/or envelope] polypeptide [(ENV)];

(b) encoding a [physiologically active and/or immunogenic] derivative [of a] polypeptide displaying a physiological and/or immunological activity substantially similar to the physiological and/or immunological activity of the polymerase (POL) as described in (a); or

(c) which is complementary to a polynucleotide sequence as defined in (a) or (b).

4. (Amended) An isolated polynucleotide fragment according to claim [1] 2:

(a) encoding the virion core polypeptide (GAG)[, polymerase (POL) and] and/or envelope polypeptide (ENV);

(b) encoding a [physiologically active and/or immunogenic] derivative [of a] polypeptide displaying a physiological and/or immunological activity of said virion core polypeptide (GAG) and/or envelope polypeptide (ENV) as described in (a); or

(c) which is complementary to a polynucleotide sequence as defined in (a) or (b).

5. (Amended) An isolated polynucleotide fragment [according to any one of claims 1 to 4 wherein the polynucleotide fragments is a deoxyribose nucleic acid (DNA) fragment] displaying at least 90% sequence identity with the sequence as shown in Figures 2 or 3:

(a) encoding the virion core polypeptide (GAG), polymerase (POL) and envelope polypeptide (ENV) of porcine retrovirus (PoEV);

(b) encoding a derivative polypeptide displaying a physiological and/or immunological activity substantially similar to the physiological and/or immunological activity of said GAG POL and ENV polypeptides as described in (a); or

(c) which is complementary to a polynucleotide sequence as defined in (a) or (b).

8. (Amended) A recombinant nucleic acid molecule comprising a polynucleotide fragment according to any one of claims 1 to [7] 5.

12. (Amended) A prokaryotic or eukaryotic host cell transformed by a polynucleotide fragment, recombinant nucleic acid molecule, or vector according to any of claims 1 to 5 and 8 to 11.

13. (Amended) A recombinant PoEV polypeptide [or derivative thereof displaying POL PoEV physiological and/or immunogenic] comprising an amino acid sequence as shown in Figure 3 displaying POL activity.

14. (Amended) A recombinant PoEV polypeptide [or derivative thereof displaying GAG and/or ENV PoEV physiological and/or immunogenic activity] comprising an amino acid sequence with at least 95% sequence identity to the GAG amino acid sequence as shown in Figure 3.

15. (Amended) A recombinant PoEV polypeptide [or derivative thereof displaying GAG, POL and ENV PoEV physiological and/or immunogenic activity] comprising an amino acid sequence with at least 75% sequence identity to the ENV amino acid sequence as shown in Figures 3 or 4.

16. (Amended) A recombinant PoEV polypeptide [according to any one of claims 13 to 15] comprising a sequence as shown in Figures 3 or 4, [or functionally active derivative thereof] or derivative polypeptide displaying a physiological and/or immunological activity of the PoEV polypeptide.

18. (Amended) An anti-PoEV antibody or fragment thereof [capable of binding to a polypeptide or fragment] raised against a polypeptide or derivative according to any one of claims 13 or 16.

19. (Amended) A polynucleotide primer which is [PoEV specific] capable of specifically hybridizing to a PoEV polynucleotide fragment as shown in figures 1, 2, 3 or 4 and capable of initiating chain extension from the 3' end of the primer, but which is not able to correctly initiate chain extension from non PoEV sequences.

20. (Amended) A polynucleotide probe which is capable of specifically [hybridising] hybridizing under stringent conditions to a [PoEV] polynucleotide sequence as shown in Figures 1, 2, 3 or 4, but not to non PoEV sequences under stringent conditions.

21. (Amended) A probe or a primer according to claims 19 or 20 which [has] have substantial nucleotide sequence identity with a strand of the molecule depicted in Figures 1, 2, 3 or 4 or a strand complementary therewith, with a corresponding RNA molecule, or with a part of such a molecule.

23. (Amended) Use of a PoEV specific polynucleotide primer or probe according to any of claims 19 to 22 in the detection of PoEV in a sample.

24. (Amended) Use of a PoEV specific polynucleotide [in a PCR] primer or primers according to either of claims 19 or 21 in a polymerase chain reaction for the detection of PoEV in a sample.

26. (Amended) Cells, tissues or organs obtainable from a pig [accoding] according to claim [25] 33.

27. (Amended) [Use of a] A recombinant PoEV polypeptide according to any one of claims 13 to 16 for use in the preparation of a vaccine.

28. (Amended) Use of an anti-PoEV antibody, [a] polynucleotide primer or probe according to any of claims 19 to 21 in the preparation of a detection kit capable of detecting the presence of PoEV nucleic acid in a sample.

29. (Amended) [Use of a] A polynucleotide; polypeptide; antibody; cells, tissues or organs according to any one of claims 1 to [7] 5, 11 to 13, [to] 16 or 26 in therapy or diagnosis.

30. (Amended) Use of a [A] polynucleotide; polypeptide; antibody; cells, tissues or organs according to anyone of claims 1 to [7] 5, 13 to 16 or 26 in the preparation of a medicament for use in therapy or diagnosis.

Please add the following claims:

32. (New) Porcine embryos, embryonic stem cells or cells containing totipotential nuclei capable of forming a viable embryo which have been manipulated by use of a polynucleotide sequence derived from the polynucleotide sequence shown in Figures 1, 2, 3 or 4 so as to substantially prevent or reduce infectious PoEV expression.

33. (New) A pig obtainable from the porcine embryos, embryonic stem cells or cells containing totipotential nuclei capable of forming a viable embryo according to claim 32.

REMARKS

In the present Amendment, Applicants have canceled claims 6, 7 and 25, have added claims 31 and 32, and amended claims 1-5, 8, 12-16, 18-21, 23-24, and 26-30. It is believed that these changes do not introduce any new matter into the claims.

A check in the amount of $2220 is enclosed to cover the national filing fees, fees for extra claims, and fees for late filing of the Declaration and Oath.

Applicants do not believe that any additional fees are required. However, if any fees, including extensions of time are required, Applicants hereby petition for same and request that the extension or other fee required for timely consideration of this application be charged to Deposit Account No. 06-1448.

Respectfully submitted,

Dated: October 19, 1998

Chinh H. Pham
Reg. No. 39,329

Patent Group
Foley, Hoag & Eliot, LLP
1 Post Office Square
Boston, Massachusetts 02109
Tel.: 617-832-1234
Fax: 617-832-1000

Porcine Retrovirus

The present invention relates inter alia to porcine retrovirus (PoEV) fragments, in particular polynucleotide fragments encoding at least one porcine retrovirus expression product, a recombinant vector comprising at least one polynucleotide fragment, use of PoEV polynucleotide fragments in the detection of native porcine retrovirus, a host cell containing at least one PoEV polynucleotide fragment or a recombinant vector comprising at least one PoEV polynucleotide fragment, PoEV polypeptides, antibodies immuno-reactive with PoEV polypeptides, pharmaceutical compositions comprising recombinant PoEV polypeptides for use as prophylactic and/or therapeutic agents and uses of PoEV polynucleotide fragments and/or polypeptides in medicine, including veterinary medicine and in the preparation of medicaments for use in medicine, including veterinary medicine.

Porcine retrovirus (PoEV) is an endogenous (genetically acquired) retrovirus isolated from pigs and expressed in cell lines derived from porcine material. There are no known pathogenic effects associated with the virus per se in its natural host although the virus appears to be associated with lymphomas in pigs and related viruses are associated with leukaemias and lymphomas in other species. The virus has been reported to infect cells from a variety of non-porcine origins and is, therefore, designated as a xenotropic, amphotropic or polytrophic virus (Lieber MM, Sherr CJ. Benveniste RE and Todaro

GJ. 1975; Strandstrom H, Verjalainen P, Moening V, Hunsmann G, Schwarz H, and Schafer W. 1974; Todaro GJ, Benveniste RE, Lieber MM and Sherr CJ. 1974). The observation that the above viruses may have the potential to infect humans and have a pathogenic effect suggests that the issue of porcine retroviruses must be addressed in the context of xenotransplanting pig tissues or cells. Therefore, information on the properties of PoEV and the development of diagnostic reagents, molecular engineering tools and potential vaccine materials is of paramount importance for example in xenotransplantation technology and the like.

It is an object of the present invention to obviate and/or mitigate against at least some of the above disadvantages.

In one aspect the present invention provides an isolated PoEV polynucleotide fragment:

(a) encoding at least one porcine retrovirus (PoEV) expression product;

(b) encoding a physiologically active and/or immunogenic derivative of said expression product; or

(c) which is complementary to a polynucleotide sequence as defined in (a) or (b).

Preferably, the polynucleotide fragment encodes the gag gene (gag), polymerase gene (*pol*) and/or envelope (*env*) gene of PoEV. Thus, said expression product can be the virion core polypeptides (GAG) and polymerase (POL) and/or envelope (ENV) polypeptides of PoEV. Thus, the invention further provides a recombinant PoEV virion core, polymerase and/or envelope polypeptide.

"Polynucleotide fragment" as used herein refers to a chain of nucleotides such as deoxyribose nucleic acid (DNA) and

transcription products thereof, such as RNA. Naturally, the skilled addressee will appreciate the whole naturally occurring PoEV genome is not included in the definition of polynucleotide fragment.

The polynucleotide fragment can be isolated in the sense that it is substantially free of biological material with which the whole genome is normally associated *in vivo*. The isolated polynucleotide fragment may be cloned to provide a recombinant molecule comprising the polynucleotide fragment. Thus, "polynucleotide fragment" includes double and single stranded DNA, RNA and polynucleotide sequences derived therefrom, for example, subsequences of said fragment and which are of any desirable length. Where a nucleic acid is single stranded then both a given strand and a sequence complementary thereto is within the scope of the present invention.

In general, the term "expression product" refers to both transcription and translation products of said polynucleotide fragments. When the expression product is a "polypeptide" (i.e. a chain or sequence of amino acids displaying a biological and/or immunological activity substantially similar to the biological and/or immunological activity of PoEV virion core, polymerase and/or envelope protein), it does not refer to a specific length of the product as such. Thus, the skilled addressee will appreciate that "polypeptide" encompasses *inter alia* peptides, polypeptides and proteins of PoEV. The polypeptide if required, can be modified *in vivo* and *in vitro*, for example by glycosylation, amidation, carboxylation, phosphorylation and/or post-translational cleavage.

Polynucleotide fragments comprising portions encompassing the PoEV genome, and derived from retrovirus particles released from a reverse transcriptase-positive porcine kidney cell line PK-15, have been molecularly cloned into a plasmid vector. This was achieved by synthesising cDNAs of PoEV RNA genomes which were recovered from porcine kidney cells expressing the endogenous virus. The cDNA was cloned into a plasmid vector and the isolated PoEV DNA fragment determined (see Figures 1,2 and 3). The sequence of the sequence identified in Figure 1 was the earliest determined sequence, followed by the sequence in Figure 2 and lastly by the most recently revised sequence shown in Figure 3. An additional study has been carried out to determine whether or not the human cell line "Raji" was susceptible to infection with the PoEV present in porcine kidney cells (PK15). A raji clone has now been obtained and the DNA sequence of its *env* gene region has been determined (see Figure 4).

The DNA fragment of Figure 3 was shown to encode three open reading frames (ORFs) of 524, 1194 and 656 amino acids respectively.

A comparison of the amino acid sequence against previously sequenced retroviruses from other species indicated that novel retrovirus cDNA had been cloned. Sequence analysis using the Lasergene software from DNASTAR Inc. showed that homologies were observed between the cloned PoEV DNA and the majority of retroviruses and that the closest homologies were to gibbon leukaemia virus (GaLV) in the polymerase (*pol*) and (*env*) regions of the pro-virus.

The first open reading frame ORF of Figure 3 (nucleotides 588-

2162) is predicted to encode the PoEV virion core polypeptide (*gag* gene). The second ORF (nucleotides 2163-5747) is predicted to encode the PoEV polymerase polypeptide (*pol* gene). The third ORF (nucleotides 5620-7590) is predicted to encode the PoEV envelope polypeptide (*env* gene). The skilled addressee will appreciate that it is possible to genetically manipulate the polynucleotide fragment or derivatives thereof, for example to clone the gene by recombinant DNA techniques generally known in the art and to express the polypeptides encoded thereby in vitro and/or in vivo. DNA fragments having the polynucleotide sequence depicted in Figures 1,2,3 and/or 4 or DNA/RNA derivatives thereof, may be used as a diagnostic tool or as a reagent for detecting PoEV nucleic acid in nucleic acids from donor animals or as a vaccine.

Preferred fragments of this aspect of the invention are polynucleotide fragments encoding: (a) at least one of the one to three polypeptides having an amino acid sequence which is shown in Figures 1,2,3 and/or 4 (b) encoding a polypeptide which is a physiologically active and/or immunogenic derivative of at least one of the polypeptides defined in (a); or (c) which is complementary to a polynucleotide sequence as defined above; or polynucleotide fragments: (a) comprising at least one of the ORFs shown in Figures 1,2,3 and/or 4 or comprising a corresponding RNA sequence; (b) comprising a sequence having substantial nucleotide sequence identity with a sequence as described in (a) above; or (c) comprising a sequence which is complementary to a sequence as described in (a) or (b) above. It is to be understood that the term "substantial sequence identity" is taken to mean at

least 50% (preferably at least 75%, at least 90%, or at least 95%) sequence identity.

The polynucleotide fragment of the present invention may be used to examine the expression and/or presence of the PoEV virus in donor animals and cells, tissues or organs derived from the donor animals to see if they are suitable for xenotransplantation (i.e. PoEV free). In addition, the recipients of pig cells, tissues or organs can be examined for the presence and/or expression of PoEV virus directly or by co-culture or infection of susceptible detector cells.

A polynucleotide fragment of the present invention may be used to identify polynucleotide sequences within the PoEV genome which are PoEV specific (i.e. it is not necessary for the complete PoEV genome to be identified). Such PoEV specific polynucleotide sequences may be used to identify PoEV nucleic acid in samples, such as transplanted cells, tissues or organs and may be included in a definitive test for PoEV.

Thus, the present invention further provides an isolated PoEV polynucleotide fragment capable of specifically hybridising to a PoEV polynucleotide sequence. In this manner, the present invention provides probes and/or primers for use in *ex vivo* and/or *in situ* PoEV virus detection and expression studies. Typical detection studies include polymerase chain reaction (PCR) studies, hybridisation studies, or sequencing studies. In principle any PoEV specific polynucleotide sequence from the above identified PoEV sequence may be used in detection and/or expression studies.

"Capable of specifically hybridising" is taken to mean that

said polynucleotide fragment preferably hybridises to a PoEV polynucleotide sequence in preference to polynucleotide sequences of other virus, animal (especially porcine or human sequences) and/or other species. In a preferment the PoEV fragment specifically binds to a native PoEV polynucleotide sequence or a part thereof.

The invention includes polynucleotide sequence(s) which are capable of specifically hybridising to a PoEV polynucleotide sequence or to a part thereof without necessarily being completely complementary to said PoEV polynucleotide sequence or fragment thereof. For example, there may be at least 50% preferably at least 75%, most preferably at least 90% or at least 95% complementarity. Of course, in some cases the sequences may be exactly complementary (100% complementary) or nearly so (e.g. there may be less than 10, preferably less than 5 mismatches). Thus, the present invention also provides anti-sense or complementary nucleotide sequence(s) which is/are capable of specifically hybridising to the disclosed DNA sequence. If a PoEV specific polynucleotide is to be used as a primer in PCR and/or sequencing studies, the polynucleotide must be capable of hybridising to PoEV nucleic acid and capable of initiating chain extension from 3' end of the polynucleotide, but not able to correctly initiate chain extension from non PoEV sequences (especially from human, or non-PoEV porcine sequences).

If a PoEV specific test polynucleotide sequence is to be used in hybridisation studies, to test for the presence of PoEV nucleic acid in a sample, the test polynucleotide should preferably remain hybridised to a sample polynucleotide under

stringent conditions. If desired, either the test or sample polynucleotide may be immobilised. Generally the test polynucleotide sequence is at least 10 or at least 50 bases in length. It may be labelled by suitable techniques known in the art. Preferably the test polynucleotide sequence is at least 200 bases in length and may even be several kilobases in length. Thus, either a denatured sample or test sequence can be first bound to a support. Hybridization can be effected at a temperature of between 50 and 70°C in double strength SSC (2xNaCl 17.5g/l and sodium citrate (SC) at 8.8g/l) buffered saline containing 0.1% sodium dodecyl sulphate (SDS). This can be followed by rinsing of the support at the same temperature but with a buffer having a reduced SSC concentration. Depending upon the degree of stringency required, and thus the degree of similarity of the sequences, such reduced concentration buffers are typically single strength SSC containing 0.1%SDS, half strength SSC containing 0.1%SDS and one tenth strength SSC containing 0.1%SDS. Sequences having the greatest degree of similarity are those the hybridisation of which is least affected by washing in buffers of reduced concentration. It is most preferred that the sample and inventive sequences are so similar that the hybridisation between them is substantially unaffected by washing or incubation in one tenth strength sodium citrate buffer containing 0.1%SDS.

PoEV specific oligonucleotides may be designed to specifically hybridise to PoEV nucleic acid. They may be synthesised, by known techniques and used as primers in PCR or sequencing reactions or as probes in hybridisations designed to

detect the presence of PoEV material in a sample. The oligonucleotides may be labelled by suitable labels known in the art, such as, radioactive labels, chemiluminescent labels or fluorescent labels and the like. Thus, the present invention also provides PoEV specific oligonucleotide probes and primers.

The term "oligonucleotide" is not meant to indicate any particular length of sequence and encompasses nucleotides of preferably at least 10b (e.g. 10b to 1kb) in length, more preferably 12b-500b in length and most preferably 15b to 100b.

The PoEV specific oligonucleotides may be determined from the PoEV sequences shown in Figure 1 and may be manufactured according to known techniques. They may have substantial sequence identity (e.g. at least 50%, at least 75%, at least 90% or at least 95% sequence identity) with one of the strands shown therein or an RNA equivalent, or with a part of such a strand. Preferably such a part is at least 10, at least 30, at least 50 or at least 200 bases long. It may be an ORF or a part thereof.

Oligonucleotides which are generally greater than 30 bases in length should preferably remain hybridised to a sample polynucleotide under one or more of the stringent conditions mentioned above. Oligonucleotides which are generally less than 30 bases in length should also preferably remain hybridised to a sample polynucleotide but under different conditions of high stringency. Typically the melting temperature of an oligonucleotide less than 30 bases may be calculated according to the formula of; 2°C for every A or T, plus 4°C for every G or C, minus 5°C. Hybridisation may take place at or around the calculated melting temperature for any particular

oligonucleotide, in 6 x SSC and 1% SDS. Non specifically hybridised oligonucleotides may then be removed by stringent washing, for example in 3 x SSC and 0.1% SDS at the same temperature. Only substantially similar matched sequences remain hybridised i.e. said oligonucleotide and corresponding PoEV nucleic acid.

When oligonucleotides of generally less than 30 bases in length are used in sequencing and/or PCR studies, the melting temperature may be calculated in the same manner as described above. The oligonucleotide may then be allowed to anneal or hybridise at a temperature around the oligonucleotides calculated melting temperature. In the case of PCR studies the annealing temperature should be around the lower of the calculated melting temperatures for the two priming oligonucleotides. It is to be appreciated that the conditions and melting temperature calculations are provided by way of example only and are not intended to be limiting. It is possible through the experience of the experimenter to vary the conditions of hybridisation and thus anneal/hybridise oligonucleotides at temperatures above their calculated melting temperature. Indeed this can be desirable in preventing so-called non-specific hybridisation from occurring.

It is possible when conducting PCR studies to predict an expected size or sizes of PCR product(s) obtainable using an appropriate combination of two or more PoEV oligonucleotides, based on where they would hybridise to the sequence in Figure 1. If, on conducting such a PCR on a sample of PoEV DNA, a fragment of the predicted size is obtained, then this is predictive that

the DNA is PoEV.

The present invention also encompasses PoEV detection kits including at least one oligonucleotide which is PoEV specific, as well as any necessary reaction reagents, washing reagents, detection reagents, signal producing agents and the like for use in the test formats outlined above.

In a further aspect there is also provided use of a PoEV specific polynucleotide in the detection of PoEV in a sample.

In a yet further aspect there is provided use of a PoEV specific polynucleotide in a PCR for the detection of PoEV in a sample.

The skilled addressee will appreciate how polynucleotide fragments may be designed and used as primers/probes in polymerase chain reaction (PCR) experiments or Southern analysis (i.e. hybridisation studies) for detecting the presence or otherwise of PoEV polynucleotide in the nucleic acid of pigs or in cell, tissue or organ samples taken from pigs (e.g. from potential transplant organs such as liver, kidney and heart). Such cells, tissues or organs can be derived from transgenic animals produced as described in EP-A-0493852, or by other means known in the art. Thus the cells, tissues or organs of transgenic pigs can be associated with one or more homologous complement restriction factors active in humans to prevent/reduce activation of complement.

Furthermore the polynucleotide fragments of the present invention can be used to analyze the genetic organisation of endogenous PoEV located in the animal cell genome in pigs thus permitting the screening of herds of pigs for altered provirus

and genomic loci (e.g. non-expressed provirus loci). Such a screening method would facilitate, for example, screening in a population of animals which are bred to lack expressed provirus and genomic loci and/or loci that do not encode infectious virus particles.

Reagents may also be developed from said polynucleotide fragments as aids to develop pigs that do not express an infectious, PoEV capable of infecting humans. Such pigs could still contain partial defective genomes that could result in the expression of non-infectious particles, viral proteins or viral mRNA. Alternatively, it may be possible to use constructs derived from the PoEV polynucleotide sequence to act as insertional mutagens to knockout the productive infectious PoEV in embryos, embryonic stem cells, or cells containing totipotential nuclei capable of forming a viable embryo. Thus *gag*, *pol* and/or *env* gene "knockouts" may be constructed to allow development of breeding programmes in pigs whereby endogenous PoEV is substantially prevented or reduced. For example the nucleotide sequence of PoEV can be manipulated e.g. by deletion of a coding sequence in vitro and the resulting construct used to replace the natural PoEV sequence by recombination. Thus, the proviral genome can be rendered inactive in the porcine cells. The knockouts can be manipulated into embryos and/or stem cells and if required manipulated nuclei can be transferred from target cells to germ cells using micromanipulation techniques well known in the art. The invention also extends to animals derived from such germ cells.

Thus, transgenic pigs may be produced containing anti-sense

constructs and/or ribozyme constructs capable of downregulating the expression of viral proteins, or transgenic pigs expressing a single chain immunoglobulin molecule with specificity for PoEV proteins or other protein that might interfere with protein synthesis or viral assembly may also be produced. Similar transgenes encoding trans-dominant negative regulators of PoEV expression or transgenes encoding competative defective "genomic RNAs" may be used to reduce or eliminate the production of infectious virions. The generation of reagents to suppress the expression of native PoEV loci in pigs, such as, by generation of antisense nucleic acids (e.g. antisense mRNAs), ribozymes or other antiviral reagents may also be developed.

The polynucleotide fragment can be molecularly cloned into a prokaryotic or eukaryotic expression vector using standard techniques and administered to a host. The expression vector is taken up by cells and the polynucleotide fragment of interest expressed, producing protein. Presentation of the protein on cell surface stimulates the host immune system to produce antibodies immunoreactive with said protein as part of a defence mechanism. Thus, expressed protein may be used as a vaccine.

Inactivated vaccines can be produced from PoEV's or cells releasing PoEV. Such infected cells may be generated by natural infection or by transfection of a proviral clone of PoEV. It will be understood that a proviral clone is a molecular clone encoding on at least one antigenic polypeptide of PoEV. After harvesting the virus and/or the infected cells, viruses or infected cells present can be inactivated for example, with formaldehyde, gluteraldehyde, acetylethylenimine or other

suitable agent or process to generate an inactivated vaccine using methods commonly employed in the art. (CVMP Working Party on Immunological Veterinary Medicinal Products (1993). General requirements for the production and control of inactivated mammalian bacterial and viral vaccines for veterinary use). Sub unit vaccines may be prepared from the individual proteins encoded by the gag, pol and env genes. Typically a vaccine would contain env gene products either alone or in combination with gag genes produced by expression in bacteria, yeast or mammlian cell systems.

Proviral clones of PoEV can be engineered to develop single cycle or replication defective viral vectors suitable for vaccination using techniques. Such viral vectors known in the art (e.g. MuLV Murine Leukaemia Retrovirus, Adenovirus and Herpesviruses (Anderson WF. (1992). Human Gene Therapy. *Science* 256, 808-813) may have one or more genes essential for replication deleted, with the missing gene function expressed constitutively or conditionally from a further, different construct which is integrated into the chromosomal DNA of a complementing cell line to the proviral PoEV clone. PoEV virions released from the cell line may infect secondary target cells in the vaccinee but not produce further infectious virus particles. For instance, the polynucleotide sequence encoding the reverse transcriptase domain of *pol* can be deleted from the proviral PoEV clone and the reverse transcriptase domain of *pol* integrated into the complementing cell line.

It will be understood that the polynucleotides; polypeptides; PoEV free cells, tissues and/or organs encompassed

by the present invention could be used in therapy, diagnosis, and/or methods of treatment. The polynucleotides; polypeptides; PoEV free cells, tissues and/or organs encompassed by the present invention can also be used in the preparation of medicaments for use in therapy or diagnosis.

The cloning and expression of a recombinant PoEV polynucleotide fragment also facilitates in producing anti-PoEV antibodies and fragments thereof (particularly monoclonal antibodies) and evaluation of in vitro and in vivo biological activity of recombinant PoEV polymerase and/or envelope polypeptides. The antibodies may be employed in diagnostic tests for native PoEV virus.

It will be understood that for the particular PoEV polypeptides embraced herein, natural variations can exist between individuals or between members of the family Suidae (i.e. the pig family). These variations may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence. All such derivatives showing active polymerase and/or envelope polypeptide physiological and/or immunological activity are included within the scope of the invention. For example, for the purpose of the present invention conservative replacements may be made between amino acids within the following groups:

(I) Alanine, serine, threonine;

(II) Glutamic acid and aspartic acid;

(III) Arginine and leucine;

(IV) Asparagine and glutamine;

(V) Isoleucine, leucine and valine;

(VI) Phenylalanine, tyrosine and tryptophan

Moreover, recombinant DNA technology may be used to prepare nucleic acid sequences encoding the various derivatives outlined above.

As is well known in the art, the degeneracy of the genetic code permits substitution of bases in a codon resulting in a different codon which is still capable of coding for the same amino acid, e.g. the codon for amino acid glutamic acid is both GAT and GAA. Consequently, it is clear that for the expression of polypeptides with the amino acid sequences shown in Figure 1 or fragments thereof, use can be made of a derivative nucleic acid sequence with such an alternative codon composition different from the nucleic acid sequence shown in said Figure 1.

Furthermore, fragments derived from the PoEV core, polymerase and/or envelope polypeptides as depicted in Figure 3, which still display PoEV virus core polypeptide, polymerase and/or envelope polypeptide properties, or fragments derived from the nucleic acid sequence encoding the virus core polypeptides, polymerase and/or envelope polypeptides or derived from the nucleotide sequence depicted in Figures 1,2,3 and/or 4encoding fragments of said virus core polypeptide, polymerase and/or envelope polypeptides are also included of the present invention. Naturally, the skilled addressee will appreciate within the ambit that the said fragments should substantially retain the physiological and/or immunological properties of the *GAG*, *POL* and/or *ENV* polypeptides.

The PoEV polynucleotide fragment of the present invention

is preferably linked to regulatory control sequences. Such control sequences may comprise promoters, operators, inducers, enhancers, ribosome binding sites, terminators etc. Suitable control sequences for a given host may be selected by those of ordinary skill in the art.

A polynucleotide fragment according to the present invention can be ligated to various expression controlling sequences, resulting in a so called recombinant nucleic acid molecule. Thus, the present invention also includes an expression vector containing an expressible PoEV nucleic acid molecule. The recombinant PoEV nucleic acid molecule can then be used for the transformation of a suitable host. Such hybrid molecules are preferably derived from, for example, plasmids or from nucleic acid sequences present in bacteriophages or viruses and are termed vector molecules.

Specific vectors which can be used to clone nucleic acid sequences according to the invention are known in the art (e.g. Rodriguez, R.L. and Denhadt, D.T., Edit., Vectors: a survey of molecular cloning vectors and their uses, Butterworths, 1988).

The methods to be used for the construction of a recombinant nucleic acid molecule according to the invention are known to those of ordinary skill in the art and are *inter alia* set forth in Sambrook, *et al.* (Molecular Cloning: a laboratory manual Cold Spring Harbour Laboratory, 1989).

The present invention also relates to a transformed cell containing the PoEV polynucleotide fragment in an expressible form. "Transformation", as used herein, refers to the introduction of a heterologous polynucleotide fragment into a

host cell. The method used may be any known in the art, for example, direct uptake, transfection transduction or electro poration (Current Protocols in Molecular Biology, 1995. John Wiley and Sons Inc). The heterologous polynucleotide fragment may be maintained through autonomous replication or alternatively, may be integrated into the host genome. The recombinant nucleic acid molecules preferably are provided with appropriate control sequences compatible with the designated host which can regulate the expression of the inserted polynucleotide fragment, e.g. tetracycline responsive promoter, thymidine kinase promoter, SV-40 promoter and the like.

Suitable hosts for the expression of recombinant nucleic acid molecules may be prokaryotic or eukaryotic in origin. Hosts suitable for the expression of recombinant nucleic acid molecules may be selected from bacteria, yeast, insect cells and mammalian cells.

Since the biological half life and the degree of glycosylation of recombinant PoEV virus core polypeptide, polymerase and/or envelope polypeptides may be important for use *in vivo*, yeast and baculovirus systems, in which a greater degree of processing and glycosylation occur, are preferred. The yeast strain *Pichia Pastoris* exhibits potential for high level expression of recombinant proteins (Clare et al., 1991). The baculovirus system has been used successfully in the production of type 1 interferons (Smith et al., 1983).

Embodiments of aspects of the present invention will now be described by way of example only which are not intended to be limiting thereof.

Examples Section

Example 1

Preparation of viral RNA

500ml of supernatant derived from exponentially growing porcine kidney cells (PK-15, American Type Culture Collection CCL 33) was clarified by centrifugation of approximately 11,000xg for 10 minutes. Virus was pelleted from the clarified supernatant by centrifugation at approximately 100,000xg for 60 minutes. The supernatant was discarded and the viral pellet retained for the preparation of viral RNA genomes. RNA was prepared from the virus pellet using a Dynabeads (registered trade mark) mRNA Direct kit according to the manufacturer's protocols; A PoEV virus pellet was resuspended in 500µl of TNE (10mM Tris HCl pH8.0, 0.1M NaCl,1mM EDTA) and the virions disrupted by the addition of 2ml of lysis/binding buffer. Dynabeads Oligo$(dT)_{25}$ were conditioned according to the manufacturer's instructions and added to the virus disrupted solution. Viral RNA was allowed to bind to the Dynabead for 10 minutes before the supernatant was removed and the bound RNA was washed three times with washing buffer with LiDS (0.5ml) and twice with washing buffer alone. The RNA was finally resuspended in 25 µl of elution solution. All procedures were performed at ambient temperature. RNase contamination was avoided by the wearing of gloves, observation of sterile technique and treatment of solutions and non-disposable glass and plasticware with diethyl pyrocarbonate (DEPC). The RNA was resuspended in DEPC- treated sterile water.

Example 2

Synthesis of cDNA

cDNA was synthesised from the purified genomic RNA using Great Lengths ™ cDNA amplification reverse transcriptase reagents (Clontech Laboratories Inc.) following the manufacturer's instructions. The RNA was primed with both oligo(dT) and random hexamers to maximise synthesis.

The Great Lengths cDNA synthesis protocol is based on a modified Gubler and Hoffman (1983) protocol for generating complementary DNA libraries and essentially consists of first-strand synthesis, second strand synthesis, adaptor ligation, and size fractionaction.

First strand synthesis: lock-docking primers anneal to the beginning of the poly-A tail of the RNA due to the presence of A, C or a residue at the 3'-end of the primer. This increases the efficiency of cDNA synthesis of eliminating unnecessary reverse transcription of long stretches of poly-A. In addition, the reverse transcriptase used is MMLV (RNase H$^-$) which gives consistently better yields than do wild-type MMLV or AMV reverse transcriptase.

Second strand synthesis: the ratio of DNA polymerase I for RNase H has been optimised to increase the efficiency of the second strand synthesis and to minimize priming by hair pin loop formation. Following secoond-strand synthesis, the ds cDNA is treated with T4 DNA polymerase to create blunt ends.

Adaptor ligation: the cDNA is ligated to a specially designed adaptor that has a pre-existing EcoRI "sticky end". The use of this adaptor, instead of a linker, eliminates the need to methylate and the EcoRI - digest the cDNA, and thus leaves internal EcoRI, sites intact. The adaptor is 5'-phosphorylated at the blunt end to allow efficient ligation to the blunt-ended cDNA.

Size fractionation: the ds cDNA is phosphorylated at the EcoRI sites and size-fractionated to remove unligated adaptors and unincorporated nucleotides. The resulting cDNA is ready for cloning into a suitable EcoRI-digested vector.

Example 3

Molecular cloning of cDNA

The size fractionated fragment was ligated with EcoR I- digested pZErO™ -1 plasmid vector DNA (Invitrogen Corporation, San Diego, U.S.). The ligation mix was used to transform competent TOP10F'cells and these were plated onto L-Agar containing zeocin following the manufacturer's instructions (Zero Background™ cloning kit - Invitrogen). Several of the resulting zeocin resistant colonies were amplified in L-Broth containing zeocin and the plasmid DNA was purified by alkaline lysis (Maniatis *et al.*, 1982).

The plasmid DNA was digested to completion with the endonuclease EcoR I and the resulting DNA fragments were separated by electrophoresis through an 1.0% agarose gel (Maniatis *et al.*, 1982), in order to check that a fragment in the

predicted size fractionated size range had been cloned. A clone identified as pPoEV was used in further experimentation.

Example 4

DNA sequence analysis.

pPoEV plasmid DNA was purified according to common techniques (Sambrook et al, 1989) and sequenced using an ABI automated sequencer. Overlapping sequencing primers from both strands of the molecular clone were used to determine the nucleotide sequence.

The first sequence obtained is shown in Figure 1. This sequence was identified as encoding two ORFs of 924 (nucleotides 23-2793) and 218 (nucleotides 2642-3297) amino acids, relating to the *pol* and *env* genes respectively. This sequence was revised and updated to the second sequence as shown in Figure 2. This second sequence was identified as encoding three ORFs of 516 (nucleotides 576-2126), 1186 (nucleotides 2143-5733) and 656 (nucleotides 5606-7576) amino acids, encoding the PoEV *gag*, *pol* and *env* genes respectively. This second sequence has since been revised and updated to the sequnce shown in Figure 3. This third sequence was identified as encoding three ORFs of 524 (nucleotides 588-2162), 1194 (nucleotides 2163-5747) and 656 (nucleotides 5620-7590) amino acids, encoding the PoEV *gag*, *pol* and *env* genes respectively.

The differences in the disclosed seqeunces is reflected by improvements in carrying out and analysing the sequence obtained. However, there is 100% identity at the nucleic acid level, between positions 21-2681 of the first sequence and positions 2972-5653 of the third sequence. Overall there is a 70.5%

identity in the entire 3310 bp of the first sequence with a corresponding portion of the third sequence.

There are only 3 base changes between the second sequence and the third sequence. These are as follows:

base no. (from Figure 2)	change
2121	insertion of a "G"
2157	insertion of a "G"
5902	"R" to an "A"
7700	"M" to an "A"

The changes at base nos. 5902 and 7700 do not effect the corresponding amino acid sequence. However, the changes at positions 2121 and 2157 alter the amino acid sequence at the end of GAG and the begining of POL. For GAG the final amino acid "S" have now been replaced by "VLALEEDKD". The total product size is now 524 amino acids. For POL, the first five amino acids "RLGET" have been deleted and replaced by "GRR". The total product size is now 1194 amino acids.

Similarities were observed between pPoEV and the majority of retroviruses determined by using alogrithims from DNASTAR Inc. Lasergene software (DNASTAR). The similarities were closest with gibbon ape leukaemia virus (GaLV) in the polymerase (*pol*) regions of the pro-virus at 68.5%, in the virus core *(gag)* region, 59.2% and in the envelope *(env)* region, 39.3% The nucleotide sequence and major ORFs of the pPoEV insert are shown in Figure 3. The largest ORF (nucleotides 2163-5747) encodes the polymerase polypeptide and the smaller ORFs (nucleotides 588-2162 and 5620-7590) encode the core and envelope polypeptides respectively.

Example 5

Purification of cellular DNA from cultured cells, tissues and blood.

Cultured cells

Cells were maintained in culture and approximately 5 x 10^7 cells were harvested for DNA preparation. The cells were pelleted by centrifugation resuspended in phosphate-buffered saline, re-centrifuged at 1000g for 2 minutes and the supernatant was discarded.

Porcine tissues

Porcine tissue samples were frozen in liquid nitrogen and powdered by grinding in a mortar or between metal foil. The samples were resuspended in 5ml of extraction buffer consisting of 0.025M EDTA (pH 8.0), 0.01MTris.Cl pH 8.0, 0.5% SDS 20μg/ml RNAse and 100μg/ml proteinase K (Maniatis *et al.*, 1982).

Porcine blood

A buffy coat was prepared from the blood samples. 20ml samples were centrifuged at 1000g for 15 minutes. The buffy coat was resuspended in buffer and the samples centrifuged at 1000g for 15 minutes. The process was repeated one further time. The sample was mixed with 5ml (3x volume) of extraction buffer (Maniatis *et al.*, 1982).

Purification

The samples (i.e. cultured cells, porcine tissue or porcine blood cells) in proteinase K-extraction buffer containing 20μg/ml RNAse

and 100μg/ml proteinase K were digested for approximately 24 hours at 37°C. The deproteinised DNA was extracted twice with phenol and twice with phenol chloroform and finally precipitated by ethanol in the presence of ammonium acetate. The DNA was recovered by centrifugation at 3000g for 30 minutes and the supernatant discarded (Maniatis *et al.*, 1982). The pellet was washed in 70% ethanol and allowed to air dry for approximately 1 hour. The DNA was allowed to re-dissolve in Tris EDTA (TE) buffer and the purity and concentration of the DNA was assessed by spectrophotometry (Maniatis *et al.*, 1982).

Example 6

Southern blot analysis of porcine tissue and cells

In order to demonstrate that the molecularly cloned DNA comprising the insert from PoEV was derived from the PK-15 cell line (American Type Culture Collection CCL33), the DNA was hybridised against cellular DNAs and its ability to detect proviral DNA was examined.

DNA purified from pPoEV was radioactively labelled and used to probe a Southern blot of endonuclease digested DNAs derived from PK-15 cells .

The DNAs probed were as follows :

a) Copy number controls of pPoEV DNA linearized by digestion with EcoRI. One copy per haploid cell genome was estimated to be 6.84pg. The control was present at an estimated copy number of 1, 5 and 10 copies.

b) PK-15 DNA.

c) Negative control HeLa (American Type Culture Collection

CCL2) DNA derived from a human adenocarcinoma cell line harbouring human papillomavirus type 18 DNA.

d) Negative control SP20 (European Collection of Animal Cell Cultures 85072401) DNA derived from a murine myeloma cell line harbouring a xenotropic MuLV retrovirus.

A hybridisation signal was observed in only the PK-15 porcine DNA. No signal was detected in either the negative human or murine DNAs. The PK-15 DNA contained more than 10 copies per cell with an estimated copy number of 20. The sizes of the three major EcoRI- endonuclease digested DNA fragments were approximately 3.8kb, 1.8kb and 0.6kb. The sizes of relevant fragments detected in the recombinant pPoEV were comparable.

There are, as expected, a number of fragments common to the genomic DNA of PK-15 and pPoEV DNA and there is agreement between the patterns observed in both DNAs digested with XhoI, BamHI and HindIII. However, there are additional fragments obtained on digestion of pPoEV DNA by a number of endonucleases.

pPoEV sequences were also detected in swine testes (American Type Culture Collection CRL 1746) and primary porcine kidney cells (Central Veterinary Laboratory batch C04495) but not in hamster CHOK1 (American Type Culture Collection CCL61) or murine NS0 myeloma cells (European Collection of Animal Cell Cultures 85110503).

In order to demonstrate that the molecularly cloned DNA comprising the insert from pPoEV could detect sequences in porcine cells and tissues in addition to PK-15 the pPoEV DNA was hybridised against cellular DNA from tissues derived from pigs and its ability to detect proviral DNA was examined (Maniatis *et al.*, 1982).

The DNA purified from pPoEV was radioactively labelled and used to probe a Southern blot of endonuclease digested DNAs derived from pig organs including liver, kidney, heart and blood.

The DNAs probed were as follows :

a) Copy number controls of pPoEV DNA linearized by digestion with EcoRI. One copy per haploid cell genome was estimated to be 6.84pg. The control was present at an estimated copy number of 5,10, 20 and 50 copies.

b) DNA purified from the porcine tissues digested with EcoRI.

A hybridisation signal was observed in all the porcine DNAs.

The DNAs contained less than 5 copies per cell. There were approximately eight distinct bands in each DNA. The sizes of the three major endonuclease digested DNA fragments were approximately 3.8kb, 1.8kb and 0.6kb.

Example 7

Polymerase Chain Reaction (PCR) Amplifications

Oligonucleotides were selected from the PoEV genome.

The upstream primer was 5′-GGA AGT GGA CTT CAC TGA G-3′.

The downstream primer was 5′-CTT TCC ACC CCG AAT CGG -3′.

The PCR was performed as described by Saiki *et al* (1987). One 1μl of 100ng/μl template DNA was added to a 49μl reaction mixture containing 200μM of dATP, dCTP, dGTP, dTTP, 30pmol of both primers from the pair described above, 1unit of DNA polymerase and 5μl of reaction buffer. The reaction buffer contained 200mM Tris-HCl pH 8.4, 500mM potassium chloride and 15mM magnesium chloride, ultrapure water. The solution was overlaid with two drops of mineral oil to prevent evaporation. Thirty five cycles of amplification were performed using a Perkin Elmer Cetus thermal cycler. Each cycle consisted of 1 minute. at 95^{0}C to denature the DNA, 1 minute. at 53^{0}C to anneal the primers to the template and 1 minute. at 72^{0}C for primer extension. After the last cycle a further incubation for 10 minutes. at 72^{0}C was performed to allow extension of any partially completed product. On completion of the amplification, 10μl of the reaction mixture was electrophoresed through a 5 per cent acrylamide gel. The DNA was visualised by staining with ethidium bromide and exposure to ultraviolet light (320nm).

The PCR reaction amplified a sequence of approximately 787bp from pPoEV and from porcine cells as expected indicating that the assay detected the PoEV proviral DNA. There was no specific amplification of the expected sequence in cells of non-porcine origin and therefore, the PCR reaction and recombinant clone can be used as a specific and sensitive diagnostic tool for detection of PoEV.

Two further digonucleotides were designed against the 3'end of the *pol* gene and s' end of the *gag* gene respectively.

The 3' *pol* oligionucleotide was 5'-GAT GGC TCT CCT GCC CTT TG-3'

The 5' *gag* oligionucleotide was 5'-CGA TGG AGG CGA AGC TTA AGG-3'

The above oligionucleotide were also used in in PCR reactions according to the conditions described above, with the exceptions that the annealing temperature was 58° and 30 cycles of replication were carried out. The PCR reaction amplified a sequence of approximately 468bp from pPoEV and from porine cells.

Example 8

Production of PoEV polypeptide in *Escherichia coli*.

The open reading frame (ORF) encoding the *pol* peptide was isolated from the pPoEV clone and molecularly cloned into the plasmid pGEX-4T-1 (Pharmacia Ltd.) for expression.

Two ml cultures of *E. coli* transformed with various expression constructs were grown with shaking at 37°C to late log phase

($O.D._{600nm}$ of 0.6) and induced by the addition of IPTG to 0.1 mM. Induced cultures were then incubated for a further 2 hours after which the bacteria were collected by centrifugation. The bacterial pellet was lysed by boiling in SDS-PAGE sample buffer and the protein profile of the induced bacteria was analysed on a 12% acrylamide gel (Laemmli, 1970) followed by staining with coomassie brilliant blue dye.

Example 9

Isolation and partial sequencing of Raji clone

The aim of the study was to determine whether the human cell line "Raji" was susceptible to infection with the PoEV present in porcine kidney cells (PK15). In order to test the capacity of the virus for xenotropism, PK15 cells were co-cultured with the B lymphoblastoid (Raji) cell line over 20 passages.

The culture system utilised direct culture and transwells, which separated the human and porcine cells, but permitted viruses to pass through the separating membrane. After every fifth passage, supernatants from the human cell lines are tested for the presence of retrovirus by reverse transcriptase assay.

Cell cultures

Porcine kidney (PK15) cells (ATCC CCL 33) were used as the source of PoEV. The human cells used for co-cultivation with PK15 cells were the lymphoblast-like Burkitts lymphoma Raji (ATCC CCL 86) cell line. This cell line does not harbour endogenous

retroviruses and lacks reverse transcriptase activity when tested by the present inventors.

Co-cultivation

Raji cells were co-cultivated directly with PK15 cells in duplicate 80cm^2 flasks and exposed to the PK15 cells throughout the 20 passage culture period. The cells were passaged twice per week and PK15 cells added as necessary from a stock culture. At every fifth passage a sample of Raji cells was removed from the co-culture, washed and cultured for 3-4 days. Supernatant was then harvested and tested for presence of retrovirus by reverse transcriptase (RT) assay.

RESULTS

The presence of reverse transcriptase activity with a preference for the Mn^{2+} cation in the supernatant from detector cell cultures is suggestive of infection by porcine retrovirus. Reverse transcriptase activity with preference for the Mn^{2+} template was not detected in the duplicate co-cultivated test cultures at passage 5 but was detected at passages 10, 15 and 20. No significant RT activity was detected in the negative control cultures. RT activity with preference for the Mn^{2+} template was detected in positive control cultures at passage 5 and 20.

An infected raji culture was diluted to single cells, and then a selection of cells cultured separately such that each culture originated from one cell. Each culture was tested by reverse-transcriptase assay.

Genomic DNA was made from an RT-positive clone as described in example 5 -purification. The PoEV ENV region was amplified by PCR as described below and the product molecularly cloned into pMOS blue T-vector (Amersham). This molecular clone was then sequenced (Fig. 4).

PCR

Oligonucleotides were selected from the PoEV genome.

The upstream primer was 5'-GAT GGC TCT CCT GCC CTT TG -3'
5' base position: 5240
The downstream primer was 5'-CCA CAG TCG TAC ACC ACG -3'
5' base position: 8144

Expected product size: 2904bp

Approx. 1 µg of genomic raji clone DNA was added to a 50 µl reaction mixture containing 200 µM of dATP, dCTP, dGTP, dTTP, 30pM each primer detailed above, 1u Taq DNA polymerase and 5µl reaction buffer. The reaction buffer contained 200mM Tris.Cl pH 8.4, 500mM potassium chloride, 15mM magnesium chloride and ultrapure water. The solution was overlaid with two drops of mineral oil to prevent evaporation. Thirty cycles of amplification was performed followed by an elongated extension reaction of 60min. at 72°C.

The cycles consisted of:

95°C 1 min.

56°C 1 min.

72°C 2 min.

The PCR product was visualised as described in example 7.

Product size: -3Kb.

CLONING

The PCR product was molecularly cloned into pMOS-Blue T-vector as directed by the manufacturer (pMOS-Blue T-vector kit - Amersham).

20 transformed colonies (clones) were picked and added to 5mls L-broth containing 50 µg/ml ampicillin. The cultures were grown shaking at 37°C overnight. Plasmid DNA was isolated from each clone using the perfect prep plasmid isolation kit as directed by the manufacturer (5 Prime - 3 Prime Inc. Boulder, CO, USA).

Plasmid DNA was digested to completion with the endonucleases EcoRI and HindIII and the products visualised on an ethidium bromide-stained 1% agarose gel. A clone (raji env clone) showing the same banding pattern as that predicted for 'PK15 cell line derived PoEV', was selected for sequencing.

SEQUENCING

Raji env clone plasmid DNA prepared above was sequenced using an ABI automated sequencer, and the commercially availableT7 sequencing primer.

The entire *env* gene region of the "Raji" was sequnced (see Figure 4) and discovered to have substantial sequence identity at both the nucleic acid and amino acid levels (98.9% and 96.3% respectively) with the PoEV sequence from PK-15.

Example 10

Phylogenetic analysis

Phylogenetic analysis was performed using the PHYLIP package. Sequence distances were calculated using the PROTDIST program (Dayhoff matrix) and a neighbour-joining unrooted phylogenetic tree reconstructed using the NEIGHBOUR program.

Bootstrapping was performed using 200 replicates of the *pol* alignment, created using the SEQBOOT program and a consensus tree was obtained using the CONSENSE program (see Figure J). The bootstrap percentages are indicated at the branch fork, with missing values equal to 100%. The data indicate that PoEV is closely related to but distinct from the type-C oncovirus typified by gibbon, murine and feline leukaemia viruses.

A phylogenetic tree was constructed from the *pol* alignment using the maximum likliehood algorithm (Dayhoff matrix). This tree differed from the *pol* NJ tree only in the placement of the BaEV lineage in relation to other mammalian type C viruses and corresponded to a low bootstrap for the BaEV fork observed in the NJ tree.

Example 11

Analysis of the LTR and adjacent region

The long terminal repeat (LTR) is a reiterated sequence at each end of the provirus that contains the enhancer and promoter governing transcription of the provirus as well as sequences required for reverse transcription of the RNA genome and integration of the proviral DNA. Three recognised domains of the LTR are identifiable, U3, R and U5 with the LTR being delineated by inverse repeats AATGAAAGG and CCTTTCATT at the 5′ and 3′ ends of U3 and U5 respectively.

LTR Domain	PoEV Genome Sequence in accordance with Figure 3	Length bp
U3	7638-8106	469
R*	8107-8188,1-61	82
U5	62-143	82

*The position of the R is defined here by similarity to the 3′end of the MuLV LTR and is compatible with the observed location of a cap site approximatelty 24 bp downstream of the TATA box.

The U3 region contans multiple potential transcription sites as shown in Figure 6. Most of the U3 region shows little or no homology to other mammalian type-C retroviruses which show conserved sites or repeat elements. However, there is homology to other mammaliann type-C viruses towards the 3′end of the U3 & region and into R and U5. Amongst the potential transcription factor sites are those for the following:

LyF-1 is a transcriptional regulator that interacts with a novel class of promoters for lymphocyte-specific-genes (Lo et al 1991).

E47 is the prototype member of a new family of tissue specific enhancer proteins that have been shown to bind to the enhancer of murine leukaemia virus.

ETS-1 is a transcription factor primarily expressed in the haematopoietic lineage.

The LTR contains direct repeats at 8006-8062 and 8045-8101 which together contain three potential CCAATT boxes. A potential TATA box is located at position 8129-8144.

The R region contains a PADS (Poly A downstream element) and consensus polyadenylation signal (AATAAA).

The primer binding site (PBS) of PoEV is glycine(2) tRNA which has not reported for any exogenous retrovirus.

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CLAIMS

1. An isolated polynucleotide fragment as shown in Figures 1, 2, 3 or 4, subsequence thereof, or corresponding RNA sequence thereof:

(a) encoding at least one porcine retrovirus (PoEV) expression product;
(b) encoding a derivative of said expression product displaying a physiological and/or immunological activity substantially similar to the physiological and/or immunological activity of said expression product as described in (a); or
(c) which is complementary to a polynucleotide sequence as defined in (a) or (b).

2. An isolated polynucleotide fragment according to claim 1:

(a) encoding at least one polypeptide having an amino acid sequence which is shown in Figures 3 or 4;
(b) encoding a derivative of said at least one polypeptide displaying a physiological and/or immunological activity substantially similar to the physiological and/or immunological activity of said expression product as described in (a); or
(c) which is complementary to a polynucleotide sequence as defined in (a) or (b).

3. An isolated polynucleotide fragment according to claim 1 or 2:

(a) encoding the polymerase (POL) polypeptide;
(b) encoding a derivative polypeptide displaying a physiological and/or immunological activity substantially similar to the physiological and/or immunological activity of the polymerase (POL) polypeptide as described in (a); or
(c) which is complementary to a polynucleotide sequence as defined in (a) or (b).

4. An isolated polynucleotide fragment according to claim 2:

(a) encoding the virion core polypeptide (GAG) and/or envelope polypeptide (ENV);
(b) encoding a derivative polypeptide displaying a physiological and/or immunological activity of said virion core polypeptide (GAG) and/or envelope polypeptide (ENV) as described in (a); or
(c) which is complementary to a polynucleotide sequence as defined in (a) or (b).

5. An isolated polynucleotide fragment displaying at least 90% sequence identity with the sequence as shown in Figures 2 or 3:

(a) encoding the virion core polypeptide (GAG), polymerase (POL) and envelope polypeptide (ENV)of porcine retrovirus (PoEV);
(b) encoding a derivative polypeptide displaying a physiological and/or immunological activity substantially similar to the physiological and/or immunological activity of said GAG POL and ENV polypeptides as described in (a); or
(c) which is complementary to a polynucleotide sequence as defined in (a) or (b).

6. A recombinant nucleic acid molecule comprising a polynucleotide fragment according to any one of claims 1 to 5.

7. A recombinant nucleic acid molecule according to claim 6 wherein the recombinant nucleic acid molecule comprises regulatory control sequences operably linked to said polynucleotide fragment for controlling expression of said polynucleotide fragment.

8. A vector comprising a recombinant nucleic acid molecule according to either of claims 6 or 7.

9. A vector according to claim 8 which is a virus or a plasmid.

10. A prokaryotic or eukaryotic host cell transformed by a polynucleotide fragment, recombinant nucleic acid molecule, or vector according to any of claims 1 to 9.

11. A recombinant PoEV polypeptide comprising an amino acid sequence as shown in Figure 3 displaying POL activity.

12. A recombinant PoEV polypeptide comprising an amino acid sequence with at least 95% sequence identify to the GAG amino acid sequence as shown in Figure 3.

13. A recombinant PoEV polypeptide comprising an amino acid sequence with at least 75% sequence identity to the ENV amino acid sequence as shown in Figures 3 or 4.

14. A recombinant PoEV polypeptide comprising a sequence as shown in Figures 3 or 4, or derivative polypeptide displaying a physiological and/or immunological activity of the PoEV polypeptide.

15. A vaccine comprising a recombinant PoEV polypeptide according to any one of claims 11 to 14, or an inactivated PoEV virus and a pharmaceutically acceptable carrier.

16. An anti-PoEV antibody or fragment thereof raised against a polypeptide or derivative according to any one of claims 11 to 14.

17. A polynucleotide primer which is capable of specifically hybridising to a PoEV polynucleotide fragment as shown in Figures 1, 2, 3 or 4 and capable of initiating chain extension from the 3' end of the primer, but which is not able to correctly initiate chain extension from non PoEV sequences.

18. A polynucleotide probe which is capable of specifically hybridising under stringent conditions to a polynucleotide sequence as shown in Figures 1, 2, 3 or 4, but not to non PoEV sequences under stringent conditions.

19. A probe or a primer according to claims 17 or 18 which have substantial nucleotide sequence identity with a strand of the molecule depicted in Figures 1, 2, 3 or 4 or a strand complementary therewith, with a corresponding RNA molecule, or with a part of such a molecule.

20. A PoEV detection kit comprising a polynucleotide primer or probe according to any of claims 17 to 19.

21. Use of a PoEV specific polynucleotide primer or probe according to any of claims 17 to 19 in the detection of PoEV in a sample.

22. Use of a PoEV specific polynucleotide primer or primers according to either of claims 17 or 19 in a polymerase chain reaction for the detection of PoEV in a sample.

23. Porcine embryos, embryonic stem cells or cells containing totipotential nuclei capable of forming a viable embryo which have been manipulated by use of a polynucleotide sequence derived from the polynucleotide sequence shown in Figures 1, 2, 3 or 4 so as to not express an infectious, PoEV.

24. A pig obtainable from the porcine embryos, embryonic stem cells or cells containing totipotentional nuclei capable of forming a viable embryo according to claim 23.

25. Cells, tissues or organs obtainable from a pig according to claim 24.

26. A recombinant PoEV polypeptide according to any one of claims 11 to 14 for use in the preparation of a vaccine.

27. Use of a polynucleotide primer or probe according to any of claims 17 to 19 in the preparation of a detection kit capable of detecting the presence of PoEV nucleic acid in a sample.

28. A polynucleotide; polypeptide; cells, tissues or organs according to any one of claims 1 to 5, 11 to 13 or 25 for use in therapy or diagnosis.

29. Use of a polynucleotide; polypeptide; cells, tissues or organs according to any one of claims 1 to 5, 11 to 13 or 25 in the preparation of a medicament for therapy or diagnosis.

30. The invention substantially as hereinbefore described.

Figure 1

```
1    GAATTCGCGGCCGCGTCGACAGATGCCTTCTTCTGCCTGAGATTACACCCCACTAGCCAA   60

61   CCACTTTTTGCCTTCGAATGGAGAGATCCAGGTACGGGAAGAACCGGGCAGCTCACCTGG  120

121  ACCCGACTGCCCCAAGGGTTCAAGAACTCCCCGACCATCTTTGACGAAGCCCTACACAGG  180

181  GACCTGGCCAACTTCAGGATCCAACACCCTCAGGTGACCCTCCTCCAGTACGTGGATGAC  240

241  CTGCTTCTGGCGGGAGCCACCAAACAGGACTGCTTAGAAGGTACGAAGGCACTACTGCTG  300

301  GAATTGTCTGACCTAGGCTACAGAGCCTCTGCTAAGAAGGCCCAGATTTGCAGGAGAGAG  360

361  GTAACATACTTGGGGTACAGTTTGCGGGGCGGGCAGCGATGGCTGACGGAGGCACGGAAG  420

421  AAAACTGTAGTCCAGATACCGGCCCCAACCACAGCCAAACAAGTGAGAGAGTTTTTGGGG  480

481  ACAGCTGGATTTTGCAGACTGTGGATCCCGGGGTTTGCGACCTTAGCAGCCCCACTCTAC  540

541  CCGCTAACCAAAGAAAAAGGGGGATTCTCCTGGGCTCCTGAGCACCAGAAGGCATTTGAT  600

601  GCTATCAAAAAGGCCCTGCTGAGCGCACCTGCTCTGGCCCTCCCTGACGTAACTAAACCC  660

661  TTTACCCTTTATGTGGATGAGCGTAAGGGAGTAGCCCGAGGAGTTTTAACCCAAACCCTA  720

721  GGACCATGGAGGAGACCTGTTGCCTACCTGTCAAAGAAGCTTGATCCTGTAGCCAGTGGT  780

781  TGGCCCGTATGTCTGAAGGCTATCGCAGCTGTGGCCATACTGGTCAAGGACGCTGACAAA  840

841  TTGACTTTGGGACAGAATATAACTGTAATAGCCCCCCATGCATTGGAGAACATCGTTCGG  900

901  CAGCCCCCAGACCGATGGATGACCAACGCCCGCATGACCCACTATCAAAGCCTGCTTCTC  960

961  ACAGAGAGGGTCACTTTCGCTCCACCAGCCGCTCTCAACCCTGCCACTCTTCTGCCTGAA  1020

1021 GAGACTGATGAACCAGTGACTCATGATTGCCATCAACTATTGATTGAGGAGACTGGGGTC  1080

1081 CGCAAGGACCTTACAGACATACCGCTGACTGGAGAAGTGCTAACCTGGTTCACTGACGGA  1140

1141 AGCAGCTATGTGGTGGAAGGTAAGAGGATGGCTGGGGCGGCAGTGGTGGACGGGACCCGC  1200

1201 ACGATCTGGGCCAGCAGCCTGCCGGAAGGAACTTCAGCGCAAAAGGCTGAGCTCATGGCC  1260
```

Figure 1 cont.

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1261  CTCACGCAAGCTTTGCGGCTGGCCGAAGGGAAATCCATAAACATTTATACGGACAGCAGG  1320

1321  TATGCCTTTGCGACTGCACACGTACACGGGGCCATCTATAAACAAAGGGGGTTGCTTACC  1380

1381  TCAGCAGGGAGGGAAATAAAGAACAAAGAGGAAATTCTAAGCCTATTAGAAGCCTTACAT  1440

1441  TTGCCAAAAAGGCTAGCTATTATACACTGTCCTGGACATCAGAAAGCCAAAGATCTCATA  1500

1501  TCTAGAGGGAACCAGATGGCTGACCGGGTTGCCAAGCAGGCAGCCCAGGCTGTTAACCTT  1560

1561  CTGCCTATAATAGAAACGCCCAAAGCCCCAGAACCCAGACGACAGTACACCCTAGAAGAC  1620

1621  TGGCAAGAGATAAAAAAAGATAGACCAGTTCTCTGAGACTCCGGAGGGGACCTGCTATACC  1680

1681  TCATATGGGAAGGAAATCCTGCCCCACAAAGAAGGGTTAGAATATGTCCAACAGATACAT  1740

1741  CGTCTAACCCACCTAGGAACTAAACACCTGCAGCAGTTGGTCAGAACATCCCCTTATCAT  1800

1801  GTTCTGAGGCTACCAGGAGTGGCTGACTCGGTGGTCAAACATTGTGTGCCCTGCCAGCTG  1860

1861  GTTAATGCTAATCCTTCCAGAATACCTCCAGGAAAGAGACTAAGGGGAAGCCACCCAGGC  1920

1921  GCTCACTGGGAAGTGGACTTCACTGAGGTAAAGCCGGCTAAATACGGAAACAAATATCTA  1980

1981  TTGGTTTTTGTAGACACCTTTTCAGGATGGGTAGAGGCTTATCCTACTAAGAAAGAGACT  2040

2041  TCAACCGTGGTGGCTAAGAAAATACTGGAGGAAATTTTTCCAAGATTTGGAATACCTAAG  2100

2101  GTAATAGGGTCAGACAATGGTCCAGCTTTCGTTGCCCAGGTAAGTCAGGGACTGGCCAAG  2160

2161  ATATTGGGGATTGATTGGAAACTGCATTGTGCATACAGACCCCAAAGCTCAGGACAGGTA  2220

2221  GAGAGGATGAATAGAACCATTAAAGAGACCCTTACCAAATTGACCACAGAGACTGGCATT  2280

2281  AATGATTGGATGGCTCTCCTGCCCTTTGTGCTTTTTAGGGTGAGGAACACCCCTGGACAG  2340

2341  TTTGGGCTGACCCCCTATGAATTGCTCTACGGGGGACCCCCCCCGTTGGCAGAAATTGCC  2400

2401  TTTGCACATAGTGCTGATGTGCTGCTTTCCCAGCCTTTGTTCTCTAGGCTCAAGGCGCTC  2460

2461  GAGTGGGTGAGGCAGCGAGCGTGGAAGCAGCTCCGGGAGGCCTACTCAGGAGGAGACTTG  2520
```

Figure 1 cont.

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2521 CAAGTTCCACATCGCTTCCAAGTTGGAGATTCAGTCTATGTTAGACGCCACCGTGCAGGA 2580

2581 AACCTCGAGACTCGGTGGAAGGGACCTTATCTCGTACTTTTGACCACACCAACGGCTGTG 2640

2641 AAAGTCGAAGGAATCCCCACCTGGATCCATGCATCCCACGTTAAGCCGGCGCCACCTCCC 2700

2701 GATTCGGGGTGGAAAGCCGAAAAGACTGAAAATCCCCTTAAGCTTCGCCTCCATCGCGTG 2760

2761 GTTCCTTACTCTGTCAATAACTCCTCAAGTTAATGGTAAACGCCTTGTGGACAGCCCGAA 2820

2821 CTCCCATAAACCCTTATCTCTCACCTGGTTACTTACTGACTCCGGTACAGGTATTAATAT 2880

2881 TAACAGCACTCAAGGGGAGGCTCCCTTGGGGACCTGGTGGCCTGAATTATATGTCTGCCT 2940

2941 TCGATCAGTAATCCCTGGTCTCAATGACCAGGCCACACCCCCCGATGTACTCCGTGCTTA 3000

3001 CGGGTTTTACGTTTGCCCAGGACCCCCAAATAATGAAGAATATTGTGGAAATCCTCAGGA 3060

3061 TTTCCTTTGCAAGCAATGGAGCTGCATAACTTCTAATGATGGGAATTGGAAATGGCCAGT 3120

3121 CTCTCAGCAAGACAGAGTAAGTTACTCTTTTGTTAACAATCCTACCAGTTATAATCAATT 3180

3181 TAATTATGGCCATGGGAGATGGAAAGATTGGCAACAGCGGGTACAAAAAGATGTACGAAA 3240

3241 TAAGCAAATAAGCTGTCATTCGTTAGACCTAGATTACTTAAAAATAAGTTTCACTAAAAA 3300

3301 AAAAAAAAAAAAAAAAAAAA 3320
```

Figure 2

```
   1 TGTGGGCCCCAGCGCGCTTGGAATAAAAATCCTCTTGCTGTTTGCATCAAGACCGCTTCT   60
  61 CGTGAGTGATTTGGGGTGTCGCCTCTTCCGAGCCCGGACGAGGGGGATTGTTCTTTTACT  120
 121 GGCCTTTCATTTGGTGCGTTGGCCGGGAAATCCTGCGACCACCCCTTACACCCGAGAACC  180
 181 GACTTGGAGGTAAAGGGATCCCCTTTGGAACGTGTGTGTGTGTCGGCCGGCGTCTCTGTT  240
 241 CTGAGTGTCTGTTTTCGGTGATGCGCGCTTTCGGTTTGCAGCTGTCCTCTCAGACCGTAA  300
 301 GGACTGGAGGACTGTGATCAGCAGACGTGCTAGGAGGATCACAGGCTGCCACCCTGGGGG  360
 361 ACGCCCCGGGAGGTGGGGAGAGCCAGGGACGCCTGGTGGTCTCCTACTGTCGGTCAGAGG  420
 421 ACCGAGTTCTGTTGTTGAAGCGAAAGCTTCCCCCTCCGCGGCCGTCCGACTCTTTTGCCT  480
 481 GCTTGTGGAAGACGCGGACGGGTCGCGTGTGTCTGGATCTGTTGGTTTCTGTCTCGTGTG  540
 541 TCTTTGTCTTGTGCGTCCTTGTCTACAGTTTTAATATGGGACAGACAGTGACTACCCCCC  600
 601 TTAGTTTGACTCTCGACCATTGGACTGAAGTTAGATCCAGGGCTCATAATTTGTCAGTTC  660
 661 AGGTTAAGAAGGGACCTTGGCAGACTTTCTGTGCCTCTGAATGGCCAACATTCGATGTTG  720
 721 GATGGCCATCAGAGGGGACCTTTAATTCTGAAATTATCCTGGCTGTTAAGGCAATCATTT  780
 781 TTCAGACTGGACCCGGCTCTCATCCTGATCAGGAGCCCTATATCCTTACGTGGCAAGATT  840
 841 TGGCAGAAGATCCTCCGCCATGGGTTAAACCATGGCTAAATAAACCAAGAAAGCCAGGTC  900
 901 CCCGAATCCTGGCTCTTGGAGAGAAAAACAAACACTCGGCCGAAAAAGTCGAGCCCTCTT  960
 961 CCTCGTATCTACCCCGAGATCGAGGAGCCGCCGACTTGGCCGGAACCCCAACCTGTTCCC 1020
1021 CCACCCCCTTATCCAGCACAGGGTGCTGTGAGGGGACCTCTGCCCCTCCTGGAGCTCCGG 1080
1081 TGGTGGAGGGACCTGCTGCCGGGACTCGGAGCCGGAGAGGCGCCACCCCGGAGCGGACAG 1140
1141 ACGAGATCGCGATATTACCGCTGCGCACCTATGGCCCTCCCATGCCAGGGGGCCAATTGC 1200
1201 AGCCCCTCCAGTATTGGCCCTTTTCTTCTGCAGATCTCTATAATTGGAAAACTAACCATC 1260
```

Figure 2 cont.

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1261 CCCCTTTCTCGGAGGATCCCCAACGCCTCACGGGGTTGGTGGAGTCCCTTATGTTCTCTC 1320
1321 ACCAGCCTACTTGGGATGATTGTCAACAGCTGCTGCAGACACTCTTCACAACCGAGGAGC 1380
1381 GAGAGAGAATTCTGTTAGAGGCTAGAAAAAATGTTCCTGGGGCCGACGGGCGACCCACGC 1440
1441 AGTTGCAAAATGAGATTGACATGGGATTTCCCTTGACTCGCCCCGGTTGGGACTACAACA 1500
1501 CGGCTGAAGGTAGGGAGAGCTTGAAAATCTATCGCCAGGCTCTGGTGGCGGGTCTCCGGG 1560
1561 GCGCCTCAAGACGGCCCACTAATTTGGCTAAGGTAAGAGAGGTGATGCAGGGACCGAACG 1620
1621 AACCTCCCTCGGTATTTCTTGAGAGGCTCATGGAAGCCTTCAGGCGGTTCACCCCTTTTG 1680
1681 ATCCTACCTCAGAGGCCCAGAAAGCCTCAGTGGCCCTGGCCTTCATTGGGCAGTCGGCTC 1740
1741 TGGATATCAGGAAGAAACTTCAGAGACTGGAAGGGTTACAGGAGGCTGAGTTACGTGATC 1800
1801 TAGTGAGAGAGGCAGAGAAGGTGTATTACAGAAGGGAGACAGAAGAGGAGAAGGAACAGA 1860
1861 GAAAAGAAAAGGAGAGAGAAGAAAGGGAGGAAAGACGTGATAGACGGCAAGAGAAGAATT 1920
1921 TGACTAAGATCTTGGCCGCAGTGGTTGAAGGGAAGAGCAGCAGGGAGAGAGAGAGAGATT 1980
1981 TTAGGAAAATTAGGTCAGGCCCTAGACAGTCAGGGAACCTGGGCAATAGGACCCCACTCG 2040
2041 ACAAGGACCAGTGTGCGTATTGTAAAGAAAAAGGACACTGGGCAAGGAACTGCCCCAAGA 2100
2101 AGGGAAACAAAGGACCGAAGTCCTAGCTCTAGAAGAAGATAAAGATTAGGGGAGACGGGT 2160
2161 TCGGACCCCCTCCCCGAGCCCAGGGTAACTTTGAAGGTGGAGGGGCAACCAGTTGAGTTC 2220
2221 CTGGTTGATACCGGAGCGGAGCATTCAGTGCTGCTACAACCATTAGGAAAACTAAAAGAA 2280
2281 AAAAAATCCTGGGTGATGGGTGCCACAGGGCAACGGCAGTATCCATGGACTACCCGAAGA 2340
2341 ACCGTTGACTTGGGAGTGGGACGGGTAACCCACTCGTTTCTGGTCATCCCTGAGTGCCCA 2400
2401 GTACCCCTTCTAGGTAGAGACTTACTGACCAAGATGGGAGCTCAAATTTCTTTTGAACAA 2460
2461 GGAAGACCAGAAGTGTCTGTGAATAACAAACCCATCACTGTGTTGACCCTCCAATTAGAT 2520
```

Figure 2 cont.

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2521 GATGAATATCGACTATATTCTCCCCAAGTAAAGCCTGATCAAGATATACAGTCCTGGTTG 2580
2581 GAGCAGTTTCCCCAAGCCTGGGCAGAAACCGCAGGGATGGGTTTGGCAAAGCAAGTTCCC 2640
2641 CCACAGGTTATTCAACTGAAGGCCAGTGCTACACCAGTATCAGTCAGACAGTACCCCTTG 2700
2701 AGTAGAGAGGCTCGAGAAGGAATTTGGCCGCATGTTCAAAGATTAATCCAACAGGGCATC 2760
2761 CTAGTTCCTGTCCAATCCCCTTGGAATACTCCCCTGCTACCGGTTAGGAAGCCTGGGACC 2820
2821 AATGATTATCGACCAGTACAGGACTTGAGAGAGGTCAATAAAAGGGTGCAGGACATACAC 2880
2881 CCAACGGTCCCGAACCCTTATAACCTCTTGAGCGCCCTCCCGCCTGAACGGAACTGGTAC 2940
2941 ACAGTATTGGACTTAAAAGATGCCTTCTTCTGCCTGAGATTACACCCCACTAGCCAACCA 3000
3001 CTTTTTGCCTTCGAATGGAGAGATCCAGGTACGGGAAGAACCGGGCAGCTCACCTGGACC 3060
3061 CGACTGCCCCAAGGGTTCAAGAACTCCCCGACCATCTTTGACGAAGCCCTACACAGGGAC 3120
3121 CTGGCCAACTTCAGGATCCAACACCCTCAGGTGACCCTCCTCCAGTACGTGGATGACCTG 3180
3181 CTTCTGGCGGGAGCCACCAAACAGGACTGCTTAGAAGGTACGAAGGCACTACTGCTGGAA 3240
3241 TTGTCTGACCTAGGCTACAGAGCCTCTGCTAAGAAGGCCCAGATTTGCAGGAGAGAGGTA 3300
3301 ACATACTTGGGGTACAGTTTGCGGGGCGGGCAGCGATGGCTGACGGAGGCACGGAAGAAA 3360
3361 ACTGTAGTCCAGATACCGGCCCCAACCACAGCCAAACAAGTGAGAGAGTTTTTGGGGACA 3420
3421 GCTGGATTTTGCAGACTGTGGATCCCGGGGTTTGCGACCTTAGCAGCCCCACTCTACCCG 3480
3481 CTAACCAAAGAAAAAGGGGGATTCTCCTGGGCTCCTGAGCACCAGAAGGCATTTGATGCT 3540
3541 ATCAAAAAGGCCCTGCTGAGCGCACCTGCTCTGGCCCTCCCTGACGTAACTAAACCCTTT 3600
3601 ACCCTTTATGTGGATGAGCGTAAGGGAGTAGCCCGAGGAGTTTTAACCCAAACCCTAGGA 3660
3661 CCATGGAGGAGACCTGTTGCCTACCTGTCAAAGAAGCTTGATCCTGTAGCCAGTGGTTGG 3720
3721 CCCGTATGTCTGAAGGCTATCGCAGCTGTGGCCATACTGGTCAAGGACGCTGACAAATTG 3780
```

Figure 2 cont.

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3781  ACTTTGGGACAGAATATAACTGTAATAGCCCCCCATGCATTGGAGAACATCGTTCGGCAG  3840

3841  CCCCCAGACCGATGGATGACCAACGCCCGCATGACCCACTATCAAAGCCTGCTTCTCACA  3900

3901  GAGAGGGTCACTTTCGCTCCACCAGCCGCTCTCAACCCTGCCACTCTTCTGCCTGAAGAG  3960

3961  ACTGATGAACCAGTGACTCATGATTGCCATCAACTATTGATTGAGGAGACTGGGGTCCGC  4020

4021  AAGGACCTTACAGACATACCGCTGACTGGAGAAGTGCTAACCTGGTTCACTGACGGAAGC  4080

4081  AGCTATGTGGTGGAAGGTAAGAGGATGGCTGGGGCGGCAGTGGTGGACGGGACCCGCACG  4140

4141  ATCTGGGCCAGCAGCCTGCCGGAAGGAACTTCAGCGCAAAAGGCTGAGCTCATGGCCCTC  4200

4201  ACGCAAGCTTTGCGGCTGGCCGAAGGGAAATCCATAAACATTTATACGGACAGCAGGTAT  4260

4261  GCCTTTGCGACTGCACACGTACACGGGGCCATCTATAAACAAAGGGGGTTGCTTACCTCA  4320

4321  GCAGGGAGGGAAATAAAGAACAAAGAGGAAATTCTAAGCCTATTAGAAGCCTTACATTTG  4380

4381  CCAAAAAGGCTAGCTATTATACACTGTCCTGGACATCAGAAAGCCAAAGATCTCATATCT  4440

4441  AGAGGGAACCAGATGGCTGACCGGGTTGCCAAGCAGGCAGCCCAGGCTGTTAACCTTCTG  4500

4501  CCTATAATAGAAACGCCCAAAGCCCCAGAACCCAGACGACAGTACACCCTAGAAGACTGG  4560

4561  CAAGAGATAAAAAAAGATAGACCAGTTCTCTGAGACTCCGGAGGGGACCTGCTATACCTCA  4620

4621  TATGGGAAGGAAATCCTGCCCCACAAAGAAGGGTTAGAATATGTCCAACAGATACATCGT  4680

4681  CTAACCCACCTAGGAACTAAACACCTGCAGCAGTTGGTCAGAACATCCCCTTATCATGTT  4740

4741  CTGAGGCTACCAGGAGTGGCTGACTCGGTGGTCAAACATTGTGTGCCCTGCCAGCTGGTT  4800

4801  AATGCTAATCCTTCCAGAATACCTCCAGGAAAGAGACTAAGGGGAAGCCACCCAGGCGCT  4860

4861  CACTGGGAAGTGGACTTCACTGAGGTAAAGCCGGCTAAATACGGAAACAAATATCTATTG  4920

4921  GTTTTTGTAGACACCTTTTCAGGATGGGTAGAGGCTTATCCTACTAAGAAAGAGACTTCA  4980

4981  ACCGTGGTGGCTAAGAAAATACTGGAGGAAATTTTTCCAAGATTTGGAATACCTAAGGTA  5040
```

Figure 2 cont.

```
5041  ATAGGGTCAGACAATGGTCCAGCTTTCGTTGCCCAGGTAAGTCAGGGACTGGCCAAGATA  5100

5101  TTGGGGATTGATTGGAAACTGCATTGTGCATACAGACCCCAAAGCTCAGGACAGGTAGAG  5160

5161  AGGATGAATAGAACCATTAAAGAGACCCTTACCAAATTGACCACAGAGACTGGCATTAAT  5220

5221  GATTGGATGGCTCTCCTGCCCTTTGTGCTTTTTAGGGTGAGGAACACCCCTGGACAGTTT  5280

5281  GGGCTGACCCCCTATGAATTGCTCTACGGGGGACCCCCCCCGTTGGCAGAAATTGCCTTT  5340

5341  GCACATAGTGCTGATGTGCTGCTTTCCCAGCCTTTGTTCTCTAGGCTCAAGGCGCTCGAG  5400

5401  TGGGTGAGGCAGCGAGCGTGGAAGCAGCTCCGGGAGGCCTACTCAGGAGGAGACTTGCAA  5460

5461  GTTCCACATCGCTTCCAAGTTGGAGATTCAGTCTATGTTAGACGCCACCGTGCAGGAAAC  5520

5521  CTCGAGACTCGGTGGAAGGGACCTTATCTCGTACTTTTGACCACACCAACGGCTGTGAAA  5580

5581  GTCGAAGGAATCCCCACCTGGATCCATGCATCCCACGTTAAGCYGGCGCCACCTCCCGAC  5640

5641  TCGGGGTGGAGAGCCGAAAAGAcTGAGAATCCCCTTAAGCTTCGCCTCCATCGCCTGGTT  5700

5701  CCTTACTCTAACAATAACTCCCCAGGCCAGTAGTAAACGCCTTATAGACAGCTCGAACCC  5760

5761  CCATAGACCTTTATCCCTTACCTGGCTGATTATTGACCCTGATACGGGTGTCACTGTAAA  5820

5821  TAGCACTCGAGGTGTTGCTCCTAGAGGCACCTGGTGGCCTGAACTGCATTTCTGCCTCCG  5880

5881  ATTGATTAACCCCGCTGTTAARAGCACACCTCCCAACCTAGTCCGTAGTTATGGGTTCTA  5940

5941  TTGCTGCCCAGGCACAGAGAAAGAGAAATACTGTGGGGGTTCTGGGGAATCCTTCTGTAG  6000

6001  GAGATGGAGCTGCGTCACCTCCAACGATGGAGACTGGAAATGGCCGATCTCTCTCCAGGA  6060

6061  CCGGGTAAAATTCTCCTTTGTCAATTCCGGCCCGGGCAAGTACAAAATGATGAAACTATA  6120

6121  TAAAGATAAGAGCTGCTCCCCATCAGACTTAGATTATCTAAAGATAAGTTTCACTGAAAG  6180

6181  GAAAACAGGAAAATATTCAAAAGTGGATAAATGGTATGAGCTGGGGAATAGTTTTTTATT  6240

6241  ATATGGCGGGGGAGCAGGGTCCACTTTAACCATTCGCCTTAGGATAGAGACGGGGACAGA  6300
```

Figure 2 cont.

```
6301  ACCCCCTGTGGCAATGGGACCCGATAAAGTACTGGCTGAACAGGGGCCCCCGGCCCTGGA  6360

6361  GCCACCGCATAACTTGCCGGTGCCCCAATTAACCTCGCTGCGGCCTGACATAACACAGCC  6420

6421  GCCTAGCAACAGTACCACTGGATTGATTCCTACCAACACGCCTAGAAACTCCCCAGGTGT  6480

6481  TCCTGTTAAGACAGGACAGAGACTCTTCAGTCTCATCCAGGGAGCTTTCCAAGCCATCAA  6540

6541  CTCCACCGACCCTGATGCCACTTCTTCTTGTTGGCTTTGTCTATCCTCAGGGCCTCCTTA  6600

6601  TTATGAGGGGATGGCTAAAGAAAGAAAATTCAATGTGACCAAAGAGCATAGAAATCAATG  6660

6661  TACATGGGGGTCCCGAAATAAGCTTACCCTCACTGAAGTTTCCGGGAAGGGGACATGCAT  6720

6721  AGGAAAAGCTCCCCCATCCCACCAACACCTTTGCTATAGTACTGTGGTTTATGAGCAGGC  6780

6781  CTCAGAAAATCAGTATTTAGTACCTGGTTATAACAGGTGGTGGGCATGCAATACTGGGTT  6840

6841  AACCCCCTGTGTTTCCACCTCAGTCTTCAACCAATCCAAAGATTTCTGTGTCATGGTCCA  6900

6901  AATCGTCCCCCGAGTGTACTACCATCCTGAGGAAGTGGTCCTTGATGAATATGACTATCG  6960

6961  GTATAACCGACCAAAAAGAGAACCCGTATCCCTTACCCTAGCTGTAATGCTCGGATTAGG  7020

7021  GACGGCCGTTGGCGTAGGAACAGGGACAGCTGCCCTGATCACAGGACCACAGCAGCTAGA  7080

7081  GAAAGGACTTGGTGAGCTACATGCGGCCATGACAGAAGATCTCCGAGCCTTAAAGGAGTC  7140

7141  TGTTAGCAACCTAGAAGAGTCCCTGACTTCTTTGTCTGAAGTGGTTCTACAGAACCGGAG  7200

7201  GGGATTAGATCTGCTGTTTCTAAGAGAAGGTGGGTTATGTGCAGCCTTAAAAGAAGAATG  7260

7261  TTGCTTCTATGTAGATCACTCAGGAGCCATCAGAGACTCCATGAACAAGCTTAGAAAAAA  7320

7321  GTTAGAGAGGCGTCGAAGGGAAAGAGAGGCTGACCAGGGGTGGTTTGAAGGATGGTTCAA  7380

7381  CAGGTCTCCTTGGATGACCACCCTGCTTTCTGCTCTGACGGGGCCCCTAGTAGTCCTGCT  7440

7441  CCTGTTACTTACAGTTGGGCCTTGCTTAATTAATAGGTTTGTTGCCTTTGTTAGAGAACG  7500

7501  AGTGAGTGCAGTCCAGATCATGGTACTTAGGCAACAGTACCAAGGCCTTCTGAGCCAAGG  7560
```

Figure 2 cont.

```
7561  AGAAACTGACCTCTAGCCTTCCCAGTTCTAAGATTAGAACTATTAACAAGACAAGAAGTG  7620

7621  GGGAATGAAAGGATGAAAATGCAACCTAACCCTCCCAGAACCCAGGAAGTTAATAAAAAG  7680

7681  CTCTAAATGCCCCCGAATTMCAGACCCTGCTGGCTGCCAGTAAATAGGTAGAAGGTCACA  7740

7741  CTTCCTATTGTTCCAGGGCCTGCTATCCTGGCCTAAGTAAGATAACAGGAAATGAGTTGA  7800

7801  CTAATCGCTTATCTGGATTCTGTAAAACTGACTGGCACCATAGAAGAATTGATTACACAT  7860

7861  TGACAGCCCTAGTGACCTATCTCAACTGCAATCTGTCACTCTGCCCAGGAGCCCACGCAG  7920

7921  ATGCGGACCTCCGGAGCTATTTTAAAATGATTGGTCCACGGAGCGCGGGCTCTCGATATT  7980

7981  TTAAAATGATTGGTCCATGGAGCGCGGGCTCTCGATATTTTAAAATGATTGGTTTGTGAC  8040

8041  GCACAGGCTTTGTTGTGAACCCCATAAAAGCTGTCCCGATTCCGCACTCGGGGCCGCAGT  8100

8101  CCTCTACCCCTGCGTGGTGTACGACTGTGGGCCCCAGCGCGCTTGGAATAAAAATCCTCT  8160

8161  TGCTGTTTGCATCAAAAAAAAAAAAAAAAAAAAA  8196
```

Figure 3

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1    GTGGTGTACGACTGTGGGCCCCAGCGCGCTTGGAATAAAAATCCTCTTGCTGTTTGCATC   60

61   AAGACCGCTTCTCGTGAGTGATTTGGGGTGTCGCCTCTTCCGAGCCCGGACGAGGGGGAT  120

121  TGTTCTTTTACTGGCCTTTCATTTGGTGCGTTGGCCGGGAAATCCTGCGACCACCCCTTA  180

181  CACCCGAGAACCGACTTGGAGGTAAAGGGATCCCCTTTGGAACGTGTGTGTGTGTCGGCC  240

241  GGCGTCTCTGTTCTGAGTGTCTGTTTTCGGTGATGCGCGCTTTCGGTTTGCAGCTGTCCT  300

301  CTCAGACCGTAAGGACTGGAGGACTGTGATCAGCAGACGTGCTAGGAGGATCACAGGCTG  360

361  CCACCCTGGGGGACGCCCCGGGAGGTGGGGAGAGCCAGGGACGCCTGGTGGTCTCCTACT  420

421  GTCGGTCAGAGGACCGAGTTCTGTTGTTGAAGCGAAAGCTTCCCCCTCCGCGGCCGTCCG  480

481  ACTCTTTTGCCTGCTTGTGGAAGACGCGGACGGGTCGCGTGTGTCTGGATCTGTTGGTTT  540

541  CTGTCTCGTGTGTCTTTGTCTTGTGCGTCCTTGTCTACAGTTTTAATATGGGACAGACAG  600
                                                    MetGlyGlnThrV

601  TGACTACCCCCCTTAGTTTGACTCTCGACCATTGGACTGAAGTTAGATCCAGGGCTCATA  660
     alThrThrProLeuSerLeuThrLeuAspHisTrpThrGluValArgSerArgAlaHisA

661  ATTTGTCAGTTCAGGTTAAGAAGGGACCTTGGCAGACTTTCTGTGCCTCTGAATGGCCAA  720
     snLeuSerValGlnValLysLysGlyProTrpGlnThrPheCysAlaSerGluTrpProT

721  CATTCGATGTTGGATGGCCATCAGAGGGGACCTTTAATTCTGAAATTATCCTGGCTGTTA  780
     hrPheAspValGlyTrpProSerGluGlyThrPheAsnSerGluIleIleLeuAlaValL

781  AGGCAATCATTTTTCAGACTGGACCCGGCTCTCATCCTGATCAGGAGCCCTATATCCTTA  840
     ysAlaIlePheGlnThrGlyProGlySerHisProAspGlnGluProTyrIleLeuT

841  CGTGGCAAGATTTGGCAGAAGATCCTCCGCCATGGGTTAAACCATGGCTAAATAAACCAA  900
     hrTrpGlnAspLeuAlaGluAspProProProTrpValLysProTrpLeuAsnLysProA

901  GAAAGCCAGGTCCCCGAATCCTGGCTCTTGGAGAGAAAAACAAACACTCGGCCGAAAAAG  960
     rgLysProGlyProArgIleLeuAlaLeuGlyGluLysAsnLysHisSerAlaGluLysV

961  TCGAGCCCTCTTCCTCGTATCTACCCCGAGATCGAGGAGCCGCCGACTTGGCCGGAACCC 1020
     alGluProSerSerSerTyrLeuProArgAspArgGlyAlaAlaAspLeuAlaGlyThrP

1021 CAACCTGTTCCCCCACCCCCTTATCCAGCACAGGGTGCTGTGAGGGGACCTCTGCCCCTC 1080
     roThrCysSerProThrProLeuSerSerThrGlyCysCysGluGlyThrSerAlaProP
```

Figure 3 cont.

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1081  CTGGAGCTCCGGTGGTGGAGGGACCTGCTGCCGGGACTCGGAGCCGGAGAGGCGCCACCC  1140
      roGlyAlaProValValGluGlyProAlaAlaGlyThrArgSerArgArgGlyAlaThrP

1141  CGGAGCGGACAGACGAGATCGCGATATTACCGCTGCGCACCTATGGCCCTCCCATGCCAG  1200
      roGluArgThrAspGluIleAlaIleLeuProLeuArgThrTyrGlyProProMetProG

1201  GGGGCCAATTGCAGCCCCTCCAGTATTGGCCCTTTTCTTCTGCAGATCTCTATAATTGGA  1260
      lyGlyGlnLeuGlnProLeuGlnTyrTrpProPheSerSerAlaAspLeuTyrAsnTrpL

1261  AAACTAACCATCCCCCTTTCTCGGAGGATCCCCAACGCCTCACGGGGTTGGTGGAGTCCC  1320
      ysThrAsnHisProProPheSerGluAspProGlnArgLeuThrGlyLeuValGluSerL

1321  TTATGTTCTCTCACCAGCCTACTTGGGATGATTGTCAACAGCTGCTGCAGACACTCTTCA  1380
      euMetPheSerHisGlnProThrTrpAspAspCysGlnGlnLeuLeuGlnThrLeuPheT

1381  CAACCGAGGAGCGAGAGAGAATTCTGTTAGAGGCTAGAAAAAATGTTCCTGGGGCCGACG  1440
      hrThrGluGluArgGluArgIleLeuLeuGluAlaArgLysAsnValProGlyAlaAspG

1441  GGCGACCCACGCAGTTGCAAAATGAGATTGACATGGGATTTCCCTTGACTCGCCCCGGTT  1500
      lyArgProThrGlnLeuGlnAsnGluIleAspMetGlyPheProLeuThrArgProGlyT

1501  GGGACTACAACACGGCTGAAGGTAGGGAGAGCTTGAAAATCTATCGCCAGGCTCTGGTGG  1560
      rpAspTyrAsnThrAlaGluGlyArgGluSerLeuLysIleTyrArgGlnAlaLeuValA

1561  CGGGTCTCCGGGGCGCCTCAAGACGGCCCACTAATTTGGCTAAGGTAAGAGAGGTGATGC  1620
      laGlyLeuArgGlyAlaSerArgArgProThrAsnLeuAlaLysValArgGluValMetG

1621  AGGGACCGAACGAACCTCCCTCGGTATTTCTTGAGAGGCTCATGGAAGCCTTCAGGCGGT  1680
      lnGlyProAsnGluProProSerValPheLeuGluArgLeuMetGluAlaPheArgArgP

1681  TCACCCCTTTTGATCCTACCTCAGAGGCCCAGAAAGCCTCAGTGGCCCTGGCCTTCATTG  1740
      heThrProPheAspProThrSerGluAlaGlnLysAlaSerValAlaLeuAlaPheIleG

1741  GGCAGTCGGCTCTGGATATCAGGAAGAAACTTCAGAGACTGGAAGGGTTACAGGAGGCTG  1800
      lyGlnSerAlaLeuAspIleArgLysLysLeuGlnArgLeuGluGlyLeuGlnGluAlaG

1801  AGTTACGTGATCTAGTGAGAGAGGCAGAGAAGGTGTATTACAGAAGGGAGACAGAAGAGG  1860
      luLeuArgAspLeuValArgGluAlaGluLysValTyrTyrArgArgGluThrGluGluG

1861  AGAAGGAACAGAGAAAAGAAAAGGAGAGAGAAGAAAGGGAGGAAAGACGTGATAGACGGC  1920
      luLysGluGlnArgLysGluLysGluArgGluGluArgGluGluArgArgAspArgArgG

1921  AAGAGAAGAATTTGACTAAGATCTTGGCCGCAGTGGTTGAAGGGAAGAGCAGCAGGGAGA  1980
      lnGluLysAsnLeuThrLysIleLeuAlaAlaValValGluGlyLysSerSerArgGluA

1981  GAGAGAGAGATTTTAGGAAAATTAGGTCAGGCCCTAGACAGTCAGGGAACCTGGGCAATA  2040
      rgGluArgAspPheArgLysIleArgSerGlyProArgGlnSerGlyAsnLeuGlyAsnA
```

Figure 3 cont

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2041  GGACCCCACTCGACAAGGACCAGTGTGCGTATTGTAAAGAAAAAGGACACTGGGCAAGGA  2100
      rgThrProLeuAspLysAspGlnCysAlaTyrCysLysGluLysGlyHisTrpAlaArgA

2101  ACTGCCCCAAGAAGGGAAACAAAGGACCGAAGgTCCTAGCTCTAGAAGAAGATAAAGATT  2160
      snCysProLysLysGlyAsnLysGlyProLysValLeuAlaLeuGluGluAspLysAspE

2161  AGGGGAGACGGGgTTCGGACCCCCTCCCCGAGCCCAGGGTAACTTTGAAGGTGGAGGGGC  2220
      ndGlyArgArgGlySerAspProLeuProGluProArgValThrLeuLysValGluGlyG

2221  AACCAGTTGAGTTCCTGGTTGATACCGGAGCGGAGCATTCAGTGCTGCTACAACCATTAG  2280
      lnProValGluPheLeuValAspThrGlyAlaGluHisSerValLeuLeuGlnProLeuG

2281  GAAAACTAAAAGAAAAAAAAATCCTGGGTGATGGGTGCCACAGGGCAACGGCAGTATCCAT  2340
      lyLysLeuLysGluLysLysSerTrpValMetGlyAlaThrGlyGlnArgGlnTyrProT

2341  GGACTACCCGAAGAACCGTTGACTTGGGAGTGGGACGGGTAACCCACTCGTTTCTGGTCA  2400
      rpThrThrArgArgThrValAspLeuGlyValGlyArgValThrHisSerPheLeuValI

2401  TCCCTGAGTGCCcAGTACCCCTTCTAGGTAGAGACTTACTGACCAAGATGGGAGCTCAAA  2460
      leProGluCysProValProLeuLeuGlyArgAspLeuLeuThrLysMetGlyAlaGlnI

2461  TTTCTTTTGAACAAGGAAGACCAGAAGTGTCTGTGAATAACAAACCCATCACTGTGTTGA  2520
      leSerPheGluGlnGlyArgProGluValSerValAsnAsnLysProIleThrValLeuT

2521  CCCTCCAATTAGATGATGAATATCGACTATATTCTCCCCAAGTAAAGCCTGATCAAGATA  2580
      hrLeuGlnLeuAspAspGluTyrArgLeuTyrSerProGlnValLysProAspGlnAspI

2581  TACAGTCCTGGTTGGAGCAGTTTCCCCAAGCCTGGGCAGAAACCGCAGGGATGGGTTTGG  2640
      leGlnSerTrpLeuGluGlnPheProGlnAlaTrpAlaGluThrAlaGlyMetGlyLeuA

2641  CAAAGCAAGTTCCCCCACAGGTTATTCAACTGAAGGCCAGTGCTACACCAGTATCAGTCA  2700
      laLysGlnValProProGlnValIleGlnLeuLysAlaSerAlaThrProValSerValA

2701  GACAGTACCCCTTGAGTAGAGAGGCTCGAGAAGGAATTTGGCCGCATGTTCAAAGATTAA  2760
      rgGlnTyrProLeuSerArgGluAlaArgGluGlyIleTrpProHisValGlnArgLeuI

2761  TCCAACAGGGCATCCTAGTTCCTGTCCAATCCCCTTGGAATACTCCCCTGCTACCGGTTA  2820
      leGlnGlnGlyIleLeuValProValGlnSerProTrpAsnThrProLeuLeuProValA

2821  GGAAGCCTGGGACCAATGATTATCGACCAGTACAGGACTTGAGAGAGGTCAATAAAAGGG  2880
      rgLysProGlyThrAsnAspTyrArgProValGlnAspLeuArgGluValAsnLysArgV

2881  TGCAGGACATACACCCAACGGTCCCGAACCCTTATAACCTCTTGAGCGCCCTCCCGCCTG  2940
      alGlnAspIleHisProThrValProAsnProTyrAsnLeuLeuSerAlaLeuProProG

2941  AACGGAACTGGTACACAGTATTGGACTTAAAAGATGCCTTCTTCTGCCTGAGATTACACC  3000
      luArgAsnTrpTyrThrValLeuAspLeuLysAspAlaPhePheCysLeuArgLeuHisP
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Figure 3 cont.

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3001  CCACTAGCCAACCACTTTTTGCCTTCGAATGGAGAGATCCAGGTACGGGAAGAACCGGGC  3060
      roThrSerGlnProLeuPheAlaPheGluTrpArgAspProGlyThrGlyArgThrGlyG

3061  AGCTCACCTGGACCCGACTGCCCCAAGGGTTCAAGAACTCCCCGACCATCTTTGACGAAG  3120
      lnLeuThrTrpThrArgLeuProGlnGlyPheLysAsnSerProThrIlePheAspGluA

3121  CCCTACACAGGGACCTGGCCAACTTCAGGATCCAACACCCTCAGGTGACCCTCCTCCAGT  3180
      laLeuHisArgAspLeuAlaAsnPheArgIleGlnHisProGlnValThrLeuLeuGlnT

3181  ACGTGGATGACCTGCTTCTGGCGGGAGCCACCAAACAGGACTGCTTAGAAGGTACGAAGG  3240
      yrValAspAspLeuLeuLeuAlaGlyAlaThrLysGlnAspCysLeuGluGlyThrLysA

3241  CACTACTGCTGGAATTGTCTGACCTAGGCTACAGAGCCTCTGCTAAGAAGGCCCAGATTT  3300
      laLeuLeuLeuGluLeuSerAspLeuGlyTyrArgAlaSerAlaLysLysAlaGlnIleC

3301  GCAGGAGAGAGGTAACATACTTGGGGTACAGTTTGCGGGGCGGGCAGCGATGGCTGACGG  3360
      ysArgArgGluValThrTyrLeuGlyTyrSerLeuArgGlyGlyGlnArgTrpLeuThrG

3361  AGGCACGGAAGAAAACTGTAGTCCAGATACCGGCCCCAACCACAGCCAAACAAGTGAGAG  3420
      luAlaArgLysLysThrValValGlnIleProAlaProThrThrAlaLysGlnValArgG

3421  AGTTTTTGGGGACAGCTGGATTTTGCAGACTGTGGATCCCGGGGTTTGCGACCTTAGCAG  3480
      luPheLeuGlyThrAlaGlyPheCysArgLeuTrpIleProGlyPheAlaThrLeuAlaA

3481  CCCCACTCTACCCGCTAACCAAAGAAAAAGGGGGATTCTCCTGGGCTCCTGAGCACCAGA  3540
      laProLeuTyrProLeuThrLysGluLysGlyGlyPheSerTrpAlaProGluHisGlnL

3541  AGGCATTTGATGCTATCAAAAAGGCCCTGCTGAGCGCACCTGCTCTGGCCCTCCCTGACG  3600
      ysAlaPheAspAlaIleLysLysAlaLeuLeuSerAlaProAlaLeuAlaLeuProAspV

3601  TAACTAAACCCTTTACCCTTTATGTGGATGAGCGTAAGGGAGTAGCCCGAGGAGTTTTAA  3660
      alThrLysProPheThrLeuTyrValAspGluArgLysGlyValAlaArgGlyValLeuT

3661  CCCAAACCCTAGGACCATGGAGGAGACCTGTTGCCTACCTGTCAAAGAAGCTTGATCCTG  3720
      hrGlnThrLeuGlyProTrpArgArgProValAlaTyrLeuSerLysLysLeuAspProV

3721  TAGCCAGTGGTTGGCCCGTATGTCTGAAGGCTATCGCAGCTGTGGCCATACTGGTCAAGG  3780
      alAlaSerGlyTrpProValCysLeuLysAlaIleAlaAlaValAlaIleLeuValLysA

3781  ACGCTGACAAATTGACTTTGGGACAGAATATAACTGTAATAGCCCCCCATGCATTGGAGA  3840
      spAlaAspLysLeuThrLeuGlyGlnAsnIleThrValIleAlaProHisAlaLeuGluA

3841  ACATCGTTCGGCAGCCCCCAGACCGATGGATGACCAACGCCCGCATGACCCACTATCAAA  3900
      snIleValArgGlnProProAspArgTrpMetThrAsnAlaArgMetThrHisTyrGlnS

3901  GCCTGCTTCTCACAGAGAGGGTCACTTTCGCTCCACCAGCCGCTCTCAACCCTGCCACTC  3960
      erLeuLeuLeuThrGluArgValThrPheAlaProProAlaAlaLeuAsnProAlaThrL
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Figure 3 cont

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3961  TTCTGCCTGAAGAGACTGATGAACCAGTGACTCATGATTGCCATCAACTATTGATTGAGG   4020
      euLeuProGluGluThrAspGluProValThrHisAspCysHisGlnLeuLeuIleGluG

4021  AGACTGGGGTCCGCAAGGACCTTACAGACATACCGCTGACTGGAGAAGTGCTAACCTGGT   4080
      luThrGlyValArgLysAspLeuThrAspIleProLeuThrGlyGluValLeuThrTrpP

4081  TCACTGACGGAAGCAGCTATGTGGTGGAAGGTAAGAGGATGGCTGGGGCGGCAGTGGTGG   4140
      heThrAspGlySerSerTyrValValGluGlyLysArgMetAlaGlyAlaAlaValValA

4141  ACGGGACCCGCACGATCTGGGCCAGCAGCCTGCCGGAAGGAACTTCAGCGCAAAAGGCTG   4200
      spGlyThrArgThrIleTrpAlaSerSerLeuProGluGlyThrSerAlaGlnLysAlaG

4201  AGCTCATGGCCCTCACGCAAGCTTTGCGGCTGGCCGAAGGGAAATCCATAAACATTTATA   4260
      luLeuMetAlaLeuThrGlnAlaLeuArgLeuAlaGluGlyLysSerIleAsnIleTyrT

4261  CGGACAGCAGGTATGCCTTTGCGACTGCACACGTACACGGGGCCATCTATAAACAAAGGG   4320
      hrAspSerArgTyrAlaPheAlaThrAlaHisValHisGlyAlaIleTyrLysGlnArgG

4321  GGTTGCTTACCTCAGCAGGGAGGGAAATAAAGAACAAAGAGGAAATTCTAAGCCTATTAG   4380
      lyLeuLeuThrSerAlaGlyArgGluIleLysAsnLysGluGluIleLeuSerLeuLeuG

4381  AAGCCTTACATTTGCCAAAAAGGCTAGCTATTATACACTGTCCTGGACATCAGAAAGCCA   4440
      luAlaLeuHisLeuProLysArgLeuAlaIleIleHisCysProGlyHisGlnLysAlaL

4441  AAGATCTCATATCTAGAGGGAACCAGATGGCTGACCGGGTTGCCAAGCAGGCAGCCCAGG   4500
      ysAspLeuIleSerArgGlyAsnGlnMetAlaAspArgValAlaLysGlnAlaAlaGlnA

4501  CTGTTAACCTTCTGCCTATAATAGAAACGCCCAAAGCCCCAGAACCCAGACGACAGTACA   4560
      laValAsnLeuLeuProIleIleGluThrProLysAlaProGluProArgArgGlnTyrT

4561  CCCTAGAAGACTGGCAAGAGATAAAAAAAGATAGACCAGTTCTCTGAGACTCCGGAGGGGA   4620
      hrLeuGluAspTrpGlnGluIleLysLysIleAspGlnPheSerGluThrProGluGlyT

4621  CCTGCTATACCTCATATGGGAAGGAAATCCTGCCCCACAAAGAAGGGTTAGAATATGTCC   4680
      hrCysTyrThrSerTyrGlyLysGluIleLeuProHisLysGluGlyLeuGluTyrValG

4681  AACAGATACATCGTCTAACCCACCTAGGAACTAAACACCTGCAGCAGTTGGTCAGAACAT   4740
      lnGlnIleHisArgLeuThrHisLeuGlyThrLysHisLeuGlnGlnLeuValArgThrS

4741  CCCCTTATCATGTTCTGAGGCTACCAGGAGTGGCTGACTCGGTGGTCAAACATTGTGTGC   4800
      erProTyrHisValLeuArgLeuProGlyValAlaAspSerValValLysHisCysValP

4801  CCTGCCAGCTGGTTAATGCTAATCCTTCCAGAATACCTCCAGGAAAGAGACTAAGGGGAA   4860
      roCysGlnLeuValAsnAlaAsnProSerArgIleProProGlyLysArgLeuArgGlyS

4861  GCCACCCAGGCGCTCACTGGGAAGTGGACTTCACTGAGGTAAAGCCGGCTAAATACGGAA   4920
      erHisProGlyAlaHisTrpGluValAspPheThrGluValLysProAlaLysTyrGlyA
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Figure 3 cont.

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4921  ACAAATATCTATTGGTTTTTGTAGACACCTTTTCAGGATGGGTAGAGGCTTATCCTACTA  4980
      snLysTyrLeuLeuValPheValAspThrPheSerGlyTrpValGluAlaTyrProThrL

4981  AGAAAGAGACTTCAACCGTGGTGGCTAAGAAAATACTGGAGGAAATTTTTCCAAGATTTG  5040
      ysLysGluThrSerThrValValAlaLysLysIleLeuGluGluIlePheProArgPheG

5041  GAATACCTAAGGTAATAGGGTCAGACAATGGTCCAGCTTTCGTTGCCCAGGTAAGTCAGG  5100
      lyIleProLysValIleGlySerAspAsnGlyProAlaPheValAlaGlnValSerGlnG

5101  GACTGGCCAAGATATTGGGGATTGATTGGAAACTGCATTGTGCATACAGACCCCAAAGCT  5160
      lyLeuAlaLysIleLeuGlyIleAspTrpLysLeuHisCysAlaTyrArgProGlnSerS

5161  CAGGACAGGTAGAGAGGATGAATAGAACCATTAAAGAGACCCTTACCAAATTGACCACAG  5220
      erGlyGlnValGluArgMetAsnArgThrIleLysGluThrLeuThrLysLeuThrThrG

5221  AGACTGGCATTAATGATTGGATGGCTCTCCTGCCCTTTGTGCTTTTTAGGGTGAGGAACA  5280
      luThrGlyIleAsnAspTrpMetAlaLeuLeuProPheValLeuPheArgValArgAsnT

5281  CCCCTGGACAGTTTGGGCTGACCCCCTATGAATTGCTCTACGGGGGACCCCCCCCGTTGG  5340
      hrProGlyGlnPheGlyLeuThrProTyrGluLeuLeuTyrGlyGlyProProProLeuA

5341  CAGAAATTGCCTTTGCACATAGTGCTGATGTGCTGCTTTCCCAGCCTTTGTTCTCTAGGC  5400
      laGluIleAlaPheAlaHisSerAlaAspValLeuLeuSerGlnProLeuPheSerArgL

5401  TCAAGGCGCTCGAGTGGGTGAGGCAGCGAGCGTGGAAGCAGCTCCGGGAGGCCTACTCAG  5460
      euLysAlaLeuGluTrpValArgGlnArgAlaTrpLysGlnLeuArgGluAlaTyrSerG

5461  GAGGAGACTTGCAAGTTCCACATCGCTTCCAAGTTGGAGATTCAGTCTATGTTAGACGCC  5520
      lyGlyAspLeuGlnValProHisArgPheGlnValGlyAspSerValTyrValArgArgH

5521  ACCGTGCAGGAAACCTCGAGACTCGGTGGAAGGGACCTTATCTCGTACTTTTGACCACAC  5580
      isArgAlaGlyAsnLeuGluThrArgTrpLysGlyProTyrLeuValLeuLeuThrThrP

5581  CAACGGCTGTGAAAGTCGAAGGAATCCCCACCTGGATCCATGCATCCCACGTTAAGCCGG  5640
      roThrAlaValLysValGluGlyIleProThrTrpIleHisAlaSerHisValLysProA
                                                    MetHisProThrLeuSerArg

5641  CGCCACCTCCCGACTCGGGGTGGAGAGCCGAAAAGACTGAGAATCCCCTTAAGCTTCGCC  5700
      laProProProAspSerGlyTrpArgAlaGluLysThrGluAsnProLeuLysLeuArgL
      ArgHisLeuProThrArgGlyGlyGluProLysArgLeuArgIleProLeuSerPheAla

5701  TCCATCGCCTGGTTCCTTACTCTAACAATAACTCCCCAGGCCAGTAGTAAACGCCTTATA  5760
      euHisArgLeuValProTyrSerAsnAsnAsnSerProGlyGlnEnd
      SerIleAlaTrpPheLeuThrLeuThrIleThrProGlnAlaSerSerLysArgLeuIle

5761  GACAGCTCGAACCCCCATAGACCTTTATCCCTTACCTGGCTGATTATTGACCCTGATACG  5820
      AspSerSerAsnProHisArgProLeuSerLeuThrTrpLeuIleIleAspProAspThr
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Figure 3 cont

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5821  GGTGTCACTGTAAATAGCACTCGAGGTGTTGCTCCTAGAGGCACCTGGTGGCCTGAACTG  5880
      GlyValThrValAsnSerThrArgGlyValAlaProArgGlyThrTrpTrpProGluLeu

5881  CATTTCTGCCTCCGATTGATTAACCCCGCTGTTAAAAGCACACCTCCCAACCTAGTCCGT  5940
      HisPheCysLeuArgLeuIleAsnProAlaValLysSerThrProProAsnLeuValArg

5941  AGTTATGGGTTCTATTGCTGCCCAGGCACAGAGAAAGAGAAATACTGTGGGGGTTCTGGG  6000
      SerTyrGlyPheTyrCysCysProGlyThrGluLysGluLysTyrCysGlyGlySerGly

6001  GAATCCTTCTGTAGGAGATGGAGCTGCGTCACCTCCAACGATGGAGACTGGAAATGGCCG  6060
      GluSerPheCysArgArgTrpSerCysValThrSerAsnAspGlyAspTrpLysTrpPro

6061  ATCTCTCTCCAGGACCGGGTAAAATTCTCCTTTGTCAATTCCGGCCCGGGCAAGTACAAA  6120
      IleSerLeuGlnAspArgValLysPheSerPheValAsnSerGlyProGlyLysTyrLys

6121  ATGATGAAACTATATAAAGATAAGAGCTGCTCCCCATCAGACTTAGATTATCTAAAGATA  6180
      MetMetLysLeuTyrLysAspLysSerCysSerProSerAspLeuAspTyrLeuLysIle

6181  AGTTTCACTGAAAGGAAAACAGGAAAATATTCAAAAGTGGATAAATGGTATGAGCTGGGG  6240
      SerPheThrGluArgLysThrGlyLysTyrSerLysValAspLysTrpTyrGluLeuGly

6241  AATAGTTTTTTATTATATGGCGGGGGAGCAGGGTCCACTTTAACCATTCGCCTTAGGATA  6300
      AsnSerPheLeuLeuTyrGlyGlyGlyAlaGlySerThrLeuThrIleArgLeuArgIle

6301  GAGACGGGGACAGAACCCCCTGTGGCAATGGGACCCGATAAAGTACTGGCTGAACAGGGG  6360
      GluThrGlyThrGluProProValAlaMetGlyProAspLysValLeuAlaGluGlnGly

6361  CCCCCGGCCCTGGAGCCACCGCATAACTTGCCGGTGCCCCAATTAACCTCGCTGCGGCCT  6420
      ProProAlaLeuGluProProHisAsnLeuProValProGlnLeuThrSerLeuArgPro

6421  GACATAACACAGCCGCCTAGCAACAGTACCACTGGATTGATTCCTACCAACACGCCTAGA  6480
      AspIleThrGlnProProSerAsnSerThrThrGlyLeuIleProThrAsnThrProArg

6481  AACTCCCCAGGTGTTCCTGTTAAGACAGGACAGAGACTCTTCAGTCTCATCCAGGGAGCT  6540
      AsnSerProGlyValProValLysThrGlyGlnArgLeuPheSerLeuIleGlnGlyAla

6541  TTCCAAGCCATCAACTCCACCGACCCTGATGCCACTTCTTCTTGTTGGCTTTGTCTATCC  6600
      PheGlnAlaIleAsnSerThrAspProAspAlaThrSerSerCysTrpLeuCysLeuSer

6601  TCAGGGCCTCCTTATTATGAGGGGATGGCTAAAGAAAGAAAATTCAATGTGACCAAAGAG  6660
      SerGlyProProTyrTyrGluGlyMetAlaLysGluArgLysPheAsnValThrLysGlu

6661  CATAGAAATCAATGTACATGGGGGTCCCGAAATAAGCTTACCCTCACTGAAGTTTCCGGG  6720
      HisArgAsnGlnCysThrTrpGlySerArgAsnLysLeuThrLeuThrGluValSerGly

6721  AAGGGGACATGCATAGGAAAAGCTCCCCCATCCCACCAACACCTTTGCTATAGTACTGTG  6780
      LysGlyThrCysIleGlyLysAlaProProSerHisGlnHisLeuCysTyrSerThrVal
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Figure 3 cont.

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6781  GTTTATGAGCAGGCCTCAGAAAATCAGTATTTAGTACCTGGTTATAACAGGTGGTGGGCA  6840
      ValTyrGluGlnAlaSerGluAsnGlnTyrLeuValProGlyTyrAsnArgTrpTrpAla

6841  TGCAATACTGGGTTAACCCCCTGTGTTTCCACCTCAGTCTTCAACCAATCCAAAGATTTC  6900
      CysAsnThrGlyLeuThrProCysValSerThrSerValPheAsnGlnSerLysAspPhe

6901  TGTGTCATGGTCCAAATCGTCCCCCGAGTGTACTACCATCCTGAGGAAGTGGTCCTTGAT  6960
      CysValMetValGlnIleValProArgValTyrTyrHisProGluGluValValLeuAsp

6961  GAATATGACTATCGGTATAACCGACCAAAAAGAGAACCCGTATCCCTTACCCTAGCTGTA  7020
      GluTyrAspTyrArgTyrAsnArgProLysArgGluProValSerLeuThrLeuAlaVal

7021  ATGCTCGGATTAGGGACGGCCGTTGGCGTAGGAACAGGGACAGCTGCCCTGATCACAGGA  7080
      MetLeuGlyLeuGlyThrAlaValGlyValGlyThrGlyThrAlaAlaLeuIleThrGly

7081  CCACAGCAGCTAGAGAAAGGACTTGGTGAGCTACATGCGGCCATGACAGAAGATCTCCGA  7140
      ProGlnGlnLeuGluLysGlyLeuGlyGluLeuHisAlaAlaMetThrGluAspLeuArg

7141  GCCTTAAAGGAGTCTGTTAGCAACCTAGAAGAGTCCCTGACTTCTTTGTCTGAAGTGGTT  7200
      AlaLeuLysGluSerValSerAsnLeuGluGluSerLeuThrSerLeuSerGluValVal

7201  CTACAGAACCGGAGGGGATTAGATCTGCTGTTTCTAAGAGAAGGTGGGTTATGTGCAGCC  7260
      LeuGlnAsnArgArgGlyLeuAspLeuLeuPheLeuArgGluGlyGlyLeuCysAlaAla

7261  TTAAAAGAAGAATGTTGCTTCTATGTAGATCACTCAGGAGCCATCAGAGACTCCATGAAC  7320
      LeuLysGluGluCysCysPheTyrValAspHisSerGlyAlaIleArgAspSerMetAsn

7321  AAGCTTAGAAAAAAGTTAGAGAGGCGTCGAAGGGAAAGAGAGGCTGACCAGGGGTGGTTT  7380
      LysLeuArgLysLysLeuGluArgArgArgArgGluArgGluAlaAspGlnGlyTrpPhe

7381  GAAGGATGGTTCAACAGGTCTCCTTGGATGACCACCCTGCTTTCTGCTCTGACGGGGCCC  7440
      GluGlyTrpPheAsnArgSerProTrpMetThrThrLeuLeuSerAlaLeuThrGlyPro

7441  CTAGTAGTCCTGCTCCTGTTACTTACAGTTGGGCCTTGCTTAATTAATAGGTTTGTTGCC  7500
      LeuValValLeuLeuLeuLeuThrValGlyProCysLeuIleAsnArgPheValAla

7501  TTTGTTAGAGAACGAGTGAGTGCAGTCCAGATCATGGTACTTAGGCAACAGTACCAAGGC  7560
      PheValArgGluArgValSerAlaValGlnIleMetValLeuArgGlnGlnTyrGlnGly

7561  CTTCTGAGCCAAGGAGAAACTGACCTCTAGCCTTCCCAGTTCTAAGATTAGAACTATTAA  7620
      LeuLeuSerGlnGlyGluThrAspLeuEnd

7621  CAAGACAAGAAGTGGGGAATGAAAGGATGAAAATGCAACCTAACCCTCCCAGAACCCAGG  7680

7681  AAGTTAATAAAAAGCTCTAAATGCCCCCGAATTACAGACCCTGCTGGCTGCCAGTAAATA  7740
```

Figure 3 cont.

```
7741  GGTAGAAGGTCACACTTCCTATTGTTCCAGGGCCTGCTATCCTGGCCTAAGTAAGATAAC  7800

7801  AGGAAATGAGTTGACTAATCGCTTATCTGGATTCTGTAAAACTGACTGGCACCATAGAAG  7860

7861  AATTGATTACACATTGACAGCCCTAGTGACCTATCTCAACTGCAATCTGTCACTCTGCCC  7920

7921  AGGAGCCCACGCAGATGCGGACCTCCGGAGCTATTTTAAAATGATTGGTCCACGGAGCGC  7980

7981  GGGCTCTCGATATTTTAAAATGATTGGTCCATGGAGCGCGGGCTCTCGATATTTTAAAAT  8040

8041  GATTGGTTTGTGACGCACAGGCTTTGTTGTGAACCCCATAAAAGCTGTCCCGATTCCGCA  8100

8101  CTCGGGGCCGCAGTCCTCTACCCCTGCGTGGTGTACGACTGTGGGCCCCAGCGCGCTTGG  8160

8161  AATAAAAATCCTCTTGCTGTTTGCATCAAAAAAAAAAAAAAAAAAAAA  8209
```

Figure 4.

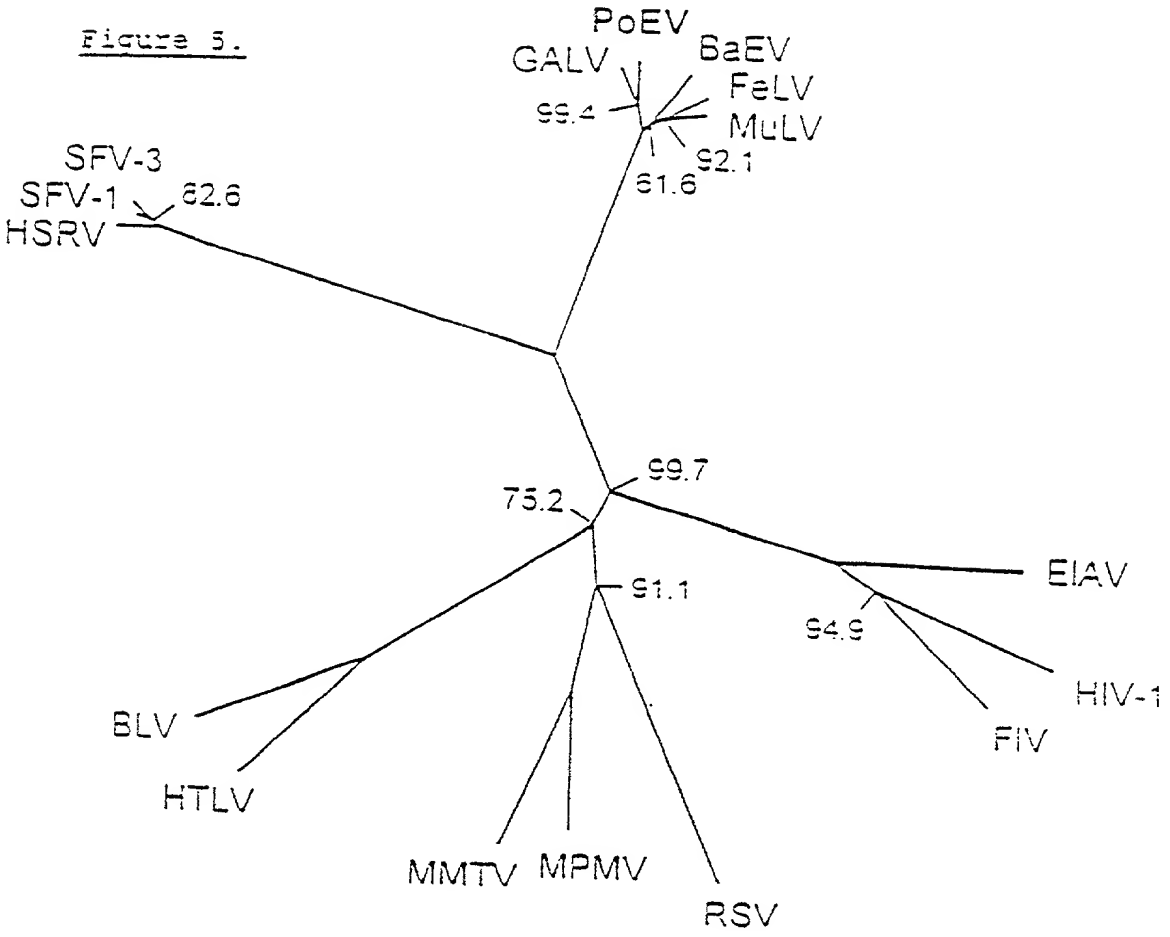
The same nucleotide sequence as represented by bases 5260 to 8210 in Figure 3 is also representative for this Figure, with the following changes:

Position	Change
5273	G-T
5341	C-T
5351	C-T
5353	T-C
5356	C-T
5426	G-A
5464	Insertion AGA
5607	C-T
5638	C-T
5792	T-C
6191	Insertion AA
6253	T-A
6255	Insertion A
6900	C-G

Such nucleotide changes result in the following amino acid changes in the ENV polypeptide.

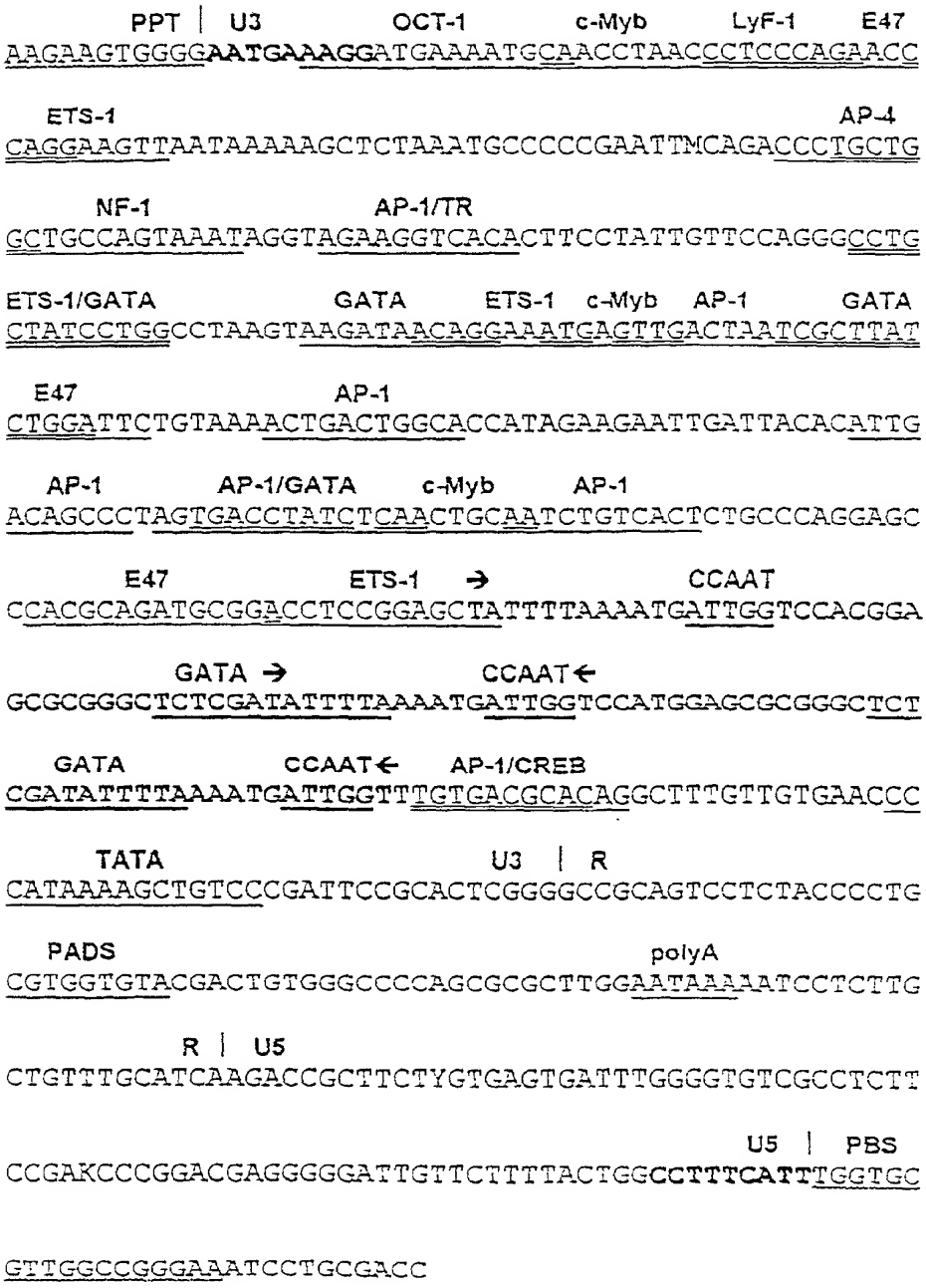
Position	Change
7	R-W
192	R-K
193	Deletion
194	Deletion
197	Y-Q
198	S-E
199	K-N
200	V-I
201	D-Q
204	Y-I
205	E-N
206	Insertions: G,M,S
206	L-W
208	N-I
209	S-V
211	L-Y
212	L-K
427	F-L

Figure 5.



MuLV murine leukaemia virus
FeLV feline leukaemia virus
GaLV gibbon ape leukaemia virus
SVV-1 simian foamy virus 1
SFV-3 simian foamy virus 3
HSRV human foamy virus
BLV Bovine leukaemia virus
HTLV human T-cell leukaemia virus
MMTV murine mammary tumour virus
MPMV Mason Pfizer monkey virus
RSV Rous sarcoma virus
FIV feline immunodeficiency virus
HIV human immunodeficiency virus
EIAV equine infectious anaemia virus

Figure 6


PPT | U3
OCT-1
c-Myb
LyF-1
E47
1 AAGAAGTGGGGAATGAAAGGATGAAAATGCAACCTAACCCTCCCAGAACC
ETS-1
AP-4
51 CAGGAAGTTAATAAAAAGCTCTAAATGCCCCCGAATTMCAGACCCTGCTG
NF-1
AP-1/TR
101 GCTGCCAGTAAATAGGTAGAAGGTCACACTTCCTATTGTTCCAGGGCCTG
ETS-1/GATA
GATA
ETS-1
c-Myb
AP-1
GATA
151 CTATCCTGGCCTAAGTAAGATAACAGGAAATGAGTTGACTAATCGCTTAT
E47
AP-1
201 CTGGATTCTGTAAAACTGACTGGCACCATAGAAGAATTGATTACACATTG
AP-1
AP-1/GATA
c-Myb
AP-1
251 ACAGCCCTAGTGACCTATCTCAACTGCAATCTGTCACTCTGCCCAGGAGC
E47
ETS-1 →
CCAAT
301 CCACGCAGATGCGGACCTCCGGAGCTATTTTAAAATGATTGGTCCACGGA
GATA →
CCAAT←
351 GCGCGGGCTCTCGATATTTTAAAATGATTGGTCCATGGAGCGCGGGCTCT
GATA
CCAAT←
AP-1/CREB
401 CGATATTTTAAAATGATTGGTTTGTGACGCACAGGCTTTGTTGTGAACCC
TATA
U3 | R
451 CATAAAAGCTGTCCCGATTCCGCACTCGGGGCCGCAGTCCTCTACCCCTG
PADS
polyA
501 CGTGGTGTACGACTGTGGGCCCCAGCGCGCTTGGAATAAAAATCCTCTTG
R | U5
551 CTGTTTGCATCAAGACCGCTTCTYGTGAGTGATTTGGGGTGTCGCCTCTT
U5 | PBS
601 CCGAKCCCGGACGAGGGGGATTGTTCTTTTACTGGCCTTTCATTGGTGC
651 GTTGGCCGGGAAATCCTGCGACC

DECLARATION FOR PATENT APPLICATION

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and sole inventor (if more than one name is listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

"PORCINE RETROVIRUS"

the specification of which (check one): ☐ is attached hereto
☐ was filed on _____ as Serial No. _____, and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with 37 CFR § 1.56(a).

Prior Foreign Application(s): I hereby claim foreign priority benefits under 35 USC § 119 of any foreign application(s) for patent or inventor's certificate listed below, and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Application No.	Country	Day/Month/Year Filed	Priority Claimed
GB9608164.1	GB	19 APRIL 1996	[x] Yes [ ] No
GB9702668.6	GB	10 FEBRUARY 97	[x] Yes [ ] No
			[ ] Yes [ ] No

I hereby appoint:

Donald W. Muirhead	Reg. No. 33,978	John C. Gorecki	Reg. No. 38,471
Beth E. Arnold	Reg. No. 35,430	Edward J. Kelly	Reg. No. 38,936
Matthew P. Vincent	Reg. No. 36,709	Sayoko Blodgett-Ford	Reg. No. P-40516
Charles H. Cella	Reg. No. 38,099		

DECLARATION FOR PATENT APPLICATION

of the Patent Group of Foley, Hoag & Eliot LLP my attorneys and/or agents to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith.

Send Correspondence to: Patent Group
Foley, Hoag & Eliot LLP
One Post Office Square
Boston, MA 02109

Direct Telephone Calls to: Matthew P. Vincent, Ph.D. (617) 832-1299

I hereby claim the benefit under 35 USC §120 of any United States application(s) or any PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by 35 USC §112, first paragraph, I acknowledge the duty to disclose material information as defined in 37 CFR §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

U.S. Application Serial No.	U.S. Filing Date	Status (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 USC §1001, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

100

Full name of sole or first inventor:	DANIEL NORMAN GALBRAITH
Inventor's signature:	Daniel Galbraith
Residence Address:	26 DON STREET, GLASGOW G33 2DH, UNITED KINGDOM GBX
Citizenship:	BRITISH
Post Office Address (if different from above):	

200

Full name of second inventor:	CHRISTINE HAWORTH
Inventor's signature:	Christine Haworth
Residence Address:	139 MARLBOROUGH AVENUE, BROOMHILL, GLASGOW G11 7JE, UNITED KINGDOM GBX
Citizenship:	BRITISH
Post Office Address (if different from above):	

Full name of third inventor:	GILLIAN MARGARET LEES
Inventor's signature:	
Residence Address:	5 KIRKVALE DRIVE, NEWTON MEARNS, GLASGOW G77 5HD, UNITED KINGDOM GBX
Citizenship:	BRITISH
Post Office Address (if different from above):	

Full name of fourth inventor:	KENNETH THOMAS SMITH
Inventor's signature:	
Residence Address:	5 KIRKVALE DRIVE, NEWTON MEARNS, GLASGOW G77 5HD, UNITED KINGDOM GBX
Citizenship:	BRITISH
Post Office Address (if different from above):	